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Production of Dimethylamine in Muscle of Several Species of Gadoid Fish during Frozen Storage, Especially in Relation to Presence of Dark Muscle

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Castell, C. H., B. Smith, and W. Neal. 1971. Production of dimethylamine in muscle of several species of gadoid fish during frozen storage, especially in relation to presence of dark muscle. J. Fish. Res. Bd. Canada 28: 1–5.

During frozen storage at –5 C, dimethylamine (DMA) was produced in the muscle of five gadoid species. The amount was lowest in haddock and increasingly higher in cod, pollock, cusk, and hake. When the dark lateral muscle was removed from the fillets before freezing, formation of DMA during frozen storage was either inhibited or greatly reduced. Under the same storage conditions no DMA was produced in the muscle of halibut, plaice, redfish, or wolffish.

Received August 17, 1970

Since the work of Reay (1939), Beatty (1938), Shewan (1938, 1939), and Beatty and Collins (1940) it has been known that small amounts of dimethylamine (DMA) are produced in the muscle of some species of fish during storage in ice. In this earlier work it was never clearly established whether the DMA was formed as the result of bacterial activity or by an enzyme native to the fish tissues. More recent work by Amano and his associates indicates that in some species of fish there is an enzyme that breaks down trimethylamine oxide to form DMA and a proportional amount of formaldehyde (Amano and Yamada 1964a, b, 1965). This activity was not confined to the muscle, but was also found in some organs of the viscera (Amano and Yamada 1965). A crude enzyme preparation has been extracted from the pyloric caeca and liver of cod but, so far, not from the muscle. It required a heat-stable cofactor (Yamada et al. 1969). This activity has been observed in tissues of squids and in several species of the family Gadidae but not in other marine animals (Amano et al. 1968; Yamada 1969).

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Although most of these Japanese experiments were carried out at 0 C and above, there is some evidence to indicate that a similar reaction occurs in some fish during frozen storage. Tokunaga (1964, 1965) has recorded the production of DMA in frozen Alaskan pollock. Castell et al. (1970) found that DMA was produced in frozen cod fillets stored at –5 and –16 C but not at –26 C; none was observed in the muscle of stored lobsters, shrimp, or scallops.

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In this present paper a more systematic examination is made of the production of DMA in nine species of commercial fish during frozen storage. By starting with strictly fresh fish, and by packaging, freezing, and storing under identical conditions, it was possible to compare the rates of DMA formation in the various species. When it was observed that the formation of DMA was greatest in the gadoid species that had the largest proportion of dark lateral muscle in the fillet, the experiments were extended to include tests with fillets in which the dark muscle had been completely excised.

Procedures

The following species of fish were used: haddock (*Melanogrammus aeglefinus*), Atlantic cod (*Gadus morhua*), pollock (*Pollachius virens*), cusk (*Brosme brosme*), silver hake (*Merluccius bilinearis*), redfish (*Sebastes marinus*), Atlantic wolffish (*Anarhichas lupus*), Atlantic halibut (*Hippoglossus hippoglossus*), and American plaice (*Hippoglossoides platessoides*). They were well-iced, trawler-caught, offshore fish in a good state of preservation at the time of filleting and all were landed in May 1970. The fillets were individually packaged in heat-sealed, laminated, nylon-polyethylene-saran pouches. In the case of very small fillets, more than one was inserted in a pouch, so that the total weight was never less than 400 g per package. They were frozen in a plate freezer for 90 min at -40 C and then stored in thermostatically controlled cold boxes at -5 C (±0.5 C). It had been found previously that storage at this temperature completely checked the growth of psychrophyllic bacteria and at the same time permitted rapid chemical changes in the frozen fish.

Total lipid content of the muscle was determined by the method of Bligh and Dyer (1959). Dimethylamine was measured by the copper-dithiocarbamate method of Dowden (1938) as modified by Dyer and Mounsey (1945). Amine values in the fish were also determined by the picric acid procedure of Dyer (1945, 1950) as well as by the modified procedure described by Tozawa et al. (1971). Dyer (1945) has pointed out that a positive reaction with the picric acid solution in his procedure occurs with both TMA and DMA, as well as with certain other methyl and ethyl amines. Tozawa et al. (1971) found that by substituting potassium hydroxide for the potassium carbonate as the alkali used in the procedure, they were able to almost completely eliminate the effect of DMA on the color reaction; this resulted in a test much more specific for TMA. Because of the uncertainty of what amines or mixtures of amines are indicated by these two procedures, the results are simply recorded as "K_2CO_3-amine" and "KOH-amine" values.

To determine whether the DMA in the frozen fillets was produced by a heat labile catalyst, some fillets were subjected to a heat treatment before freezing. This was done by sealing them in plastic bags in the usual manner, placing them in flowing steam for 5 min, and then autoclaving them for 10 min at 110 C (between 5 and 6 lb steam pressure). Examination of the fillets immediately after this heat treatment showed an 80% reduction in extractable protein; they had a typical cooked fish odour and appearance and had extruded considerable moisture. Following defrosting after storage, the moisture that had been extruded from the fillets was included in the sample for analyses.

Results

LIPID CONTENT

The average lipid contents, expressed as % of the total weight of the fillet, were: haddock 0.60, cusk 0.61, cod 0.67, flounder 0.75, halibut 0.85, redfish 0.86, pollock 0.87, hake 0.97, and wolffish 3.46. The lipid content of the flounder and redfish, though lower than at other times of the year for these species, was within the range for these fish when caught in May. With the exception of the wolffish, they were all within the category that is generally described as lean fish.

DIMETHYLAMINE

There was no DMA production in the fillets of flounder, halibut, wolffish, or redfish. The gadoids, on the other hand, all produced DMA, although the amounts varied greatly with the different species (Fig. 1). The smallest amounts were produced in the haddock muscle, slightly more in the cod, and increasingly larger amounts in the pollock, cusk, and hake.

TRIMETHYLAMINE

Use of the modification of Dyer's (1945, 1950) colorimetric picric acid test proposed by Tozawa and his associates (1971) indicated that little or no TMA was formed during storage (Fig. 2). It is interesting to compare these curves with those obtained by the original picric acid procedure (Fig. 3) and the direct measurements of DMA (Fig. 1). There is a striking similarity between the curves for each of the species in Fig. 1 and 3. This clearly indicates that the values obtained with the unmodified picric acid test are almost wholly from the DMA that has been produced during storage.

SIGNIFICANCE OF THE DARK LATERAL MUSCLE IN PRODUCTION OF DMA IN FILLETS

It was observed that hake fillets, which produced the most DMA, had the largest proportion of dark muscle; and for the gadoids as a group there appeared to be a correlation between the amounts of DMA formed and the amounts of dark muscle in the fillets. When the dark muscle in hake fillets was cut away before freezing there was a marked inhibition of DMA production during storage.

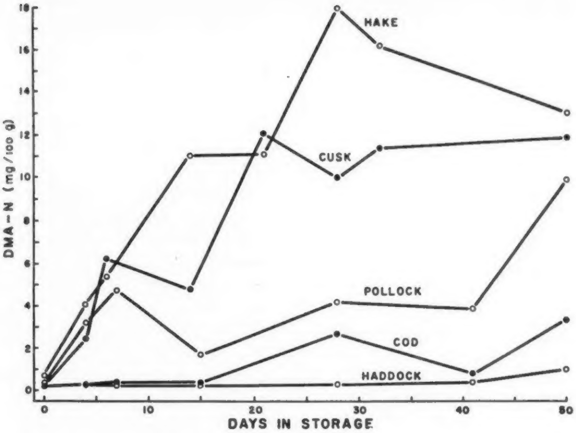

FIG. 1. DMA values of haddock, cod, pollock, cusk, and hake fillets that were frozen in a plate freezer at –40 C for 90 min and then stored for 50 days at –5 C.

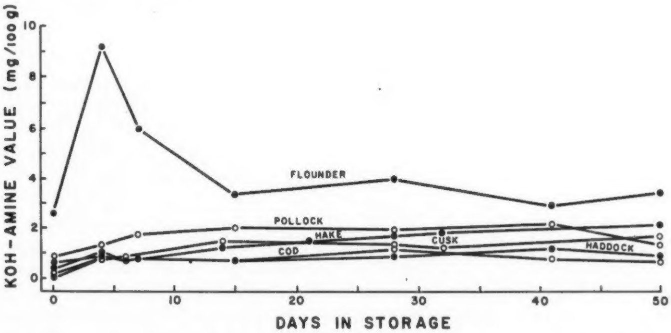

FIG. 2. Amine values of fillets used in Fig. 1, using Tozawa et al.'s (1971) modification of Dyer's (1945) picric acid procedure. This modification has been reported to eliminate the effect of any DMA that may be present in the fish.

This is shown in the following values (mg/100 g) for storage periods up to 35 days:

Days in storage	0	2	5	8	14	26	35
Whole fillets	6.3	11.9	10.3	22.5	22.1	24.5	30.5
White muscle	2.5	2.5	2.5	1.6	4.5	4.5	9.5

A slightly different experiment was carried out with cod fillets. Instead of comparing the whole fillets with white muscle only, the cod fillets were cut into two parts: one consisted of white muscle only and the other, the remaining portion of the fillet, contained the dark muscle and some of the adjoining white muscle still adhering to it. The DMA values (mg/100 g) for these were as follows:

Days in storage	0	4	15	27
White muscle	0.3	0.5	1.7	2.9
Dark + white muscle	1.2	4.9	6.8	9.6

A third set of experiments was carried out by freezing whole hake fillets in the usual manner. After 69 days of storage the fillets were defrosted and then separated into white muscle and the remaining portion of the fillet, which was predominantly dark muscle but also had some of the adjoining white muscle still adhering to it. The DMA values for these two fractions were: white muscle only 30.3; dark muscle plus some adjoining white muscle 78.9.

EFFECT OF HEATING FILLETS BEFORE FREEZING

Heating the fillets (5 min steaming followed by 10 min autoclaving at 110 C) reduced the pro-

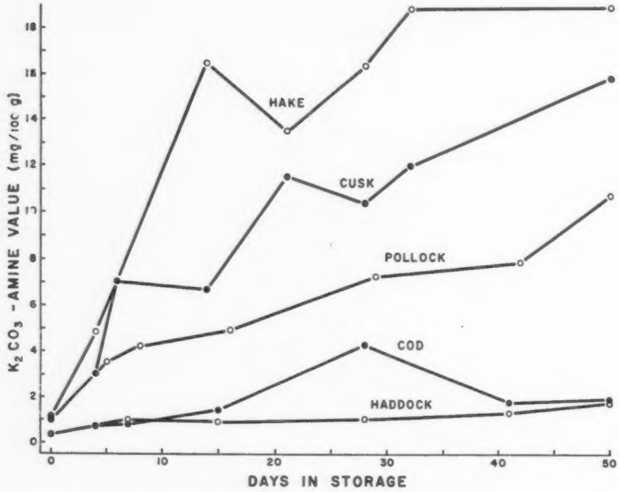

FIG. 3. Amine values of fillets used in Fig. 1 and 2, obtained with Dyer's colorimetric picric acid procedure. These values include both DMA and TMA.

duction of DMA during frozen storage but did not eliminate it. Results from a typical example carried out with hake fillets gave the following DMA values (mg/100 g):

Days in storage	0	5	8	14	26	30
Uncooked fillets	6.2	10.3	22.5	22.0	27.5	30.5
Cooked fillets	2.0	4.8	8.9	12.2	14.5	15.2

The results with cod were not so striking and required longer storage periods because of the smaller amounts of DMA that were involved:

Days in storage	0	5	10	36	103
Uncooked fillets	0.06	0.80	1.67	4.9	9.2
Cooked fillets	>0.06	0.25	0.46	0.6	1.9

This suggests that if the production of DMA is the result of an enzymic activity, the enzymes concerned are quite heat resistant.

Discussion

These results confirm the findings of Amano, Yamada, and their associates in Japan that nonmicrobial formation of DMA in fish tissues is a characteristic of the gadoid species. In an extensive review of their work, Yamada et al. (1969) stated that more than 45 species were examined and that, DMA formation was demonstrated in only 8 of them, all of these being in the cod family. Tokunaga (1964) followed the formation of DMA in frozen Alaska pollock and showed that the rate of its formation was a function of the storage temperature, being rapid at –2 C and decreasing as the temperature was lowered to –25 C. Most of the other studies have been carried out on unfrozen fish preparations at temperatures ranging between 0 and 10 C.

Our interest has been in commercial species of fish caught in the Atlantic off the coast of Nova Scotia. It has been confined to a study of the development of DMA in whole fillets frozen at –40 C and stored at –5 C. This temperature is low enough to inhibit bacterial activity and high enough to permit rapid development of DMA in the muscle. We encountered no DMA in four nongadoid species (halibut, plaice, redfish, and wolffish), and have previously reported no DMA formation in frozen lobster, shrimp, and scallop muscle (Castell et al. 1970). Preliminary tests with shad and mackerel (unpublished data) have indicated that small amounts of TMA, but little or no DMA, are formed in these fatty fish during frozen storage.

Not only was DMA found in each of the gadoid species that was examined, but the quantities of DMA produced were characteristic of the species: the amounts were small in cod and haddock, and increasingly larger in pollock, cusk, and hake.

So far as we are aware it has not been previously reported that the dark lateral muscle is an important factor in the production of DMA. This is of particular interest, because in the next paper in this series we will report that, in addition to reducing DMA formation, removal of the dark lateral muscle before freezing brings about other chemical changes in the spoilage pattern of gadoid fillets during frozen storage. Amano and Yamada (1965) found that some of the visceral organs of cod, pollock, and hake were more active than muscle in the production of DMA. One cannot help but wonder whether some of the heme pigments, which are abundant in the dark muscle and in many of the internal organs, may be involved. Our results with the precooked fish suggest that whatever the catalyst may be it is heat stable.

The question has been asked: are the results reported here, with only one batch of fish for each species, typical of what might be expected if the tests were repeated periodically throughout the year? Results from incompleted periodical tests indicate that there are seasonal variations in the DMA-producing activity of the muscle but the same seasonal trend applies to all the species that have been tested. For example, repeated experiments with haddock, cod, and hake have shown more rapid development, and production of larger amounts, of DMA in the summer and fall than in the spring.

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Seasonal Changes in the Chemical Composition and Food Reserves of the Freshwater Triclad *Dugesia tigrina* (Platyhelminthes: Turbellaria)

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BODDINGTON, M. J., AND D. F. METTRICK. 1971. Seasonal changes in the chemical composition and food reserves of the freshwater triclad *Dugesia tigrina* (Platyhelminthes: Turbellaria). J. Fish. Res. Bd. Canada 28: 7–14.

Changes in the food reserves and chemical constituents of a population of *Dugesia tigrina* (Girard, 1850) from Grenadier Pond, Toronto, Ont., have been followed over a 13-month period from October 1968 through October 1969. The parameters examined were individual worm dry weight, protein nitrogen, nonprotein nitrogen, lipid nitrogen, *lyo*-glycogen, *desmo*-glycogen, and total lipid.

Whereas the nitrogen, lipid, and *desmo*-glycogen fractions remained relatively constant when expressed on a weight-to-unit-weight basis, irrespective of changes in the number and size of individual worms, the *lyo*-glycogen fraction decreased exponentially with an increase in the number of worms in the population. This decrease mirrored the relation between the size of individual worms and the number of worms in the population. The negative regression coefficient of *lyo*-glycogen on population biomass was statistically significant ($P < 0.05$).

The changes at different times of the year in the chemical constituents and food reserves of *D. tigrina* have been correlated with variations resulting from the life cycle of *D. tigrina* in both the size of the population and the size of the individual members comprising the population.

The results are consistent with Reynoldson's (1966) hypothesis that triclad populations show restricted fluctuations in numbers because the population size is regulated by intraspecific competition for a limited food source. The significance of a limited food resource is demonstrated by showing that, as the population size increased, intraspecific competition resulted in decreases in the *lyo*-glycogen food reserves at both the individual and population level.

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LITTLE of the work on North American triclads relates to the ecology of the species in any terms other than the physical factors affecting distribution (Jenkins and Miller 1962; Stokely et al. 1965; Whitehead MS 1965; Chandler 1966; Mettrick et al. 1970). In Europe Reynoldson and his coworkers have completed extensive studies on the ecology of triclads, and considered such factors as population dynamics, food resource involved, distribution of the food species and its correlation to the population biology of the flatworms, and influence of predators on population structure.

Much of this information was reviewed by Reynoldson (1966a) who concluded, first, that triclad populations show restricted fluctuations in numbers because they live under conditions of food shortage for long periods of the year; second, that the numbers in the population are regulated by intraspecific competition for food; and third, that the ability of these worms to reabsorb and regenerate tissue as required and their high degree of immunity to predators increases the importance of intraspecific competition for food in the regulation of population size.

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No comparable work has been completed in North America, although Root (1960) and Armstrong (1964) have done some laboratory studies on the population dynamics of *Dugesia tigrina*, and Maguire (1962, 1963) has studied the possible effect of ectocrines on the reproduction of *Cura foremanii.*

In nutritional studies carried out on mammals (Munro 1964) and echinoderms (Geise 1966), changes in the chemical composition of an organism have been used as an index of the animal's nutritional state. In view of the emphasis that Reynoldson (1966a) has placed upon food as a limiting factor in population dynamics of European triclads, changes in chemical composition may also be applied to the testing of his hypothesis, since if available food is a limiting factor in population structure of triclads, this will be reflected in food reserves present in the organism.

The objectives of the present investigation were, therefore, to demonstrate seasonal changes in food reserves of a triclad population, and to determine if variation in food reserves was related to changes in individual worm and population size.

With lack of information relevant to North America on such aspects as food, predators, and the population biology of the species present, support for Reynoldson's hypothesis would be significant in a much wider sphere, as it would suggest a general applicability to the North American fauna of hypotheses based on studies on European triclads.

Materials and Methods

A large population of the triclad *D. tigrina* (Girard, 1850) was found in Grenadier Pond, High Park, Toronto, Ont. This lake covers a surface area of 50 acres and is approximately $\frac{3}{4} \times \frac{1}{4}$ ml. *Dugesia tigrina* was found in great numbers on stones, rocks, and other debris along the southern margin of the lake, over a shore length of approximately 500 yards where there is a littoral shelf varying from 1 to 2 yards in width before the bottom slopes sharply into deeper waters.

All samples for chemical analysis were taken from this collection site. Collections, starting in October 1968 through October 1969, were made on a biweekly basis to reduce the possibility of affecting the structure of the population by overcropping, although because such a large area was available for the collection of the samples, and the number of worms present was large (see Table 1 and Discussion), such a possibility was considered to be unlikely.

A sample of 100–200 triclads was collected by hand, washed several times in pond water, the remaining debris removed by means of a pasteur pipette, and the worms counted.

BIOCHEMICAL METHODS

Wherever possible, worms were handled by means of a pasteur pipette to minimise the loss of mucus, which may be considerable if forceps, brush, or spatula are utilised. Reduction of mucus loss is important, as mucus production may account for nearly 70% of the worm's energy intake (Teal 1957). The worm sample was transferred to a preweighed planchet, excess water was removed, and the sample weighed after standing for 20 min so that the last traces of water evaporated. After weighing, the worm sample was dried for 24 hr at 100 C, reweighed, and stored at −30 C.

EXTRACTION OF TOTAL LIPIDS

The dried tissue was reconstituted to its original fresh weight by the addition of distilled water, and the sample then homogenised at 0 C in a Potter–Elvehjem grinding flask with chloroform:methanol (2:1).

The lipids were extracted by a modification of the biphasic extraction procedure of Folch et al. (1957). The modifications were: first, ×5 the volumes of the extractants used, second, the use of centrifugation at 0 C as opposed to filtration; and third, the retention of all liquids and solids superfluous to the lipid extraction for later estimation of carbohydrate and nitrogen content.

EXTRACTION WITH TRICHLOROACETIC ACID (NONPROTEIN NITROGEN: LYO-GLYCOGEN)

The precipitate, upper phase, and washing superfluous to the Folch et al. (1957) lipid analysis were reconstituted. An aliquot of this homogenate was added to 0.5 volume of 15% ice-cold trichloroacetic acid (TCA), and the suspension was allowed to stand for 10 min with occasional stirring. Subsequent treatment followed the procedure of Mettrick and Jennings (1969). Nonprotein nitrogen was estimated by a micro-kjeldahl procedure, following Fleck and Munro (1964). *Lyo*-glycogen was estimated with anthrone reagent following the procedure outlined by Trevelyan and Harrison (1952).

ALKALINE HYDROLYSIS (PROTEIN NITROGEN: DESMO-GLYCOGEN)

The insoluble TCA residue was dissolved in 2–5 ml 0.3 N potassium hydroxide (KOH), and hydrolyzed for 1 hr at 37 C.

Aliquots of this solution were removed for estimation of protein nitrogen (tissue nitrogen) by micro-kjeldahl determination. Further aliquots were taken to estimate *desmo*-glycogen with anthrone reagent.

Results

The changes in the protein nitrogen, nonprotein nitrogen, lipid nitrogen, *lyo*-glycogen, *desmo*-glycogen, and total lipid components of *D. tigrina* over the period October 1968 through October 1969, are shown in Table 1. The results are expressed as micrograms/milligram worm dry weight.

The total of the three nitrogen fractions remained relatively constant over the entire experimental period (90.16 ± 5.00). The means ± standard error for the protein nitrogen, nonprotein nitrogen, and lipid nitrogen components were 70.5 ± 3.5, 18.1 ± 1.6, and 2.2 ± 0.1 μg/mg worm dry weight respectively.

The variations in the protein nitrogen and nonprotein nitrogen fractions over the 13-month period were not proportional, as is shown by the changes in the ratios of nonprotein:protein nitrogen.

The two carbohydrate fractions showed considerably more variation during the experimental period than that shown by the two major nitrogen fractions.

TABLE 1. Changes in the size and chemical composition of a population of *Dugesia tigrina* over the 13-month period October 1968 through October 1969.

	1968				1969											
	Oct.		Nov.		Apr.	May		June		July		Aug.		Sept.		Oct.
	15	23	1	14	25	9	23	5	19	16	25	11	20	4	18	3
Population size																
No. present (*worms collected/hr*)	350	200	110	50	60	120	180	250	400	1400	2400	500	300	150	100	60
Avg worm size (*μg dry wt*)	592.0	651.5	435.0	335.0	353.0	927.0	469.9	880.0	473.0	159.0	116.0	210.0	209.0	284.0	278.0	560.0
Chemical composition																
Lipid nitrogen (*μg/mg dry wt*)	2.6	2.2	2.4	1.6	0.7	2.4	2.1	2.5	3.5	2.1	4.5	2.9	2.5	1.1	1.1	1.1
Protein nitrogen (*μg/mg dry wt*)	57.9	64.1	72.3	72.8	65.4	50.2	49.6	87.9	81.7	105.5	56.9	74.4	70.1	64.7	67.1	76.9
Nonprotein nitrogen (*μg/mg dry wt*)	10.2	13.2	12.5	15.4	28.8	24.5	17.7	13.8	17.2	15.8	26.1	23.8	23.4	17.3	26.5	13.6
Ratio of nonprotein nitrogen:protein nitrogen (*1*:-)	5.7	4.9	5.8	4.7	2.2	2.0	2.8	6.4	4.7	6.7	2.2	3.1	3.0	3.7	2.5	5.7
Lyo-glycogen (*μg/mg dry wt*)	103.5	73.5	58.4	93.6	27.3	100.2	84.6	52.3	35.2	21.2	37.6	40.3	39.4	32.3	41.3	61.4
Desmo-glycogen (*μg/mg dry wt*)	41.0	46.4	70.4	40.9	17.3	33.6	30.4	74.3	86.2	46.3	39.7	59.9	65.9	10.1	28.3	62.2
Ratio of *desmo*-:*lyo*-glycogen (*1*:-)	2.5	1.6	0.8	2.3	1.6	2.9	2.8	0.7	0.4	0.5	0.9	0.7	0.6	3.2	1.5	0.9
Total lipid (*μg/mg dry wt*)	212.6	221.5	216.3	175.0	201.0	151.1	179.5	159.8	180.1	169.3	163.0	187.4	192.9	204.6	174.8	185.8

These differences were reflected in the *desmo-:lyo*-glycogen ratio, which varied from 1:0.4 in June to 1:3.2 in September.

The total lipid fraction remained relatively constant throughout the experimental period, with an overall mean value of 185.9 ± 5.3 µg/mg of dried worm tissue.

These results show the changes occurring in the chemical constituents per unit dry weight of the population, but they do not take into account the fact that the population structure changes in relation to the life cycle of *D. tigrina*. Analyses of the food reserves of the population may mask changes in the chemical composition of individual worms at different times of the year.

In Fig. 1 changes in the protein-nitrogen and nonprotein nitrogen fractions with time are expressed as micrograms per individual worm averaged from the sample, hereafter referred to as micrograms per mean individual. These variations are considerably greater than those shown by the population as a whole.

Both nitrogen fractions (Fig. 1) showed increases in early May, but whereas the nonprotein nitrogen component then decreased and remained constant for the rest of the year, the protein-nitrogen fraction reached a second larger peak in June, declined rapidly in July, and increased again in September and October 1969, to mirror a similar increase in October 1968.

Changes in the *lyo*- and *desmo*-glycogen fractions, expressed as micrograms/mean individual worm, are shown in Fig. 2.

In early spring, carbohydrate food reserves per individual were minimal. There were rapid increases in both fractions during April, prior to the onset of the breeding period, that coincided with the fall in glycogen reserves in May. There was a further increase in both *lyo*- and *desmo*-glycogen in early June, and a rapid decline in late June. Glycogen food reserves increased in September–October 1969, and matched a similar rise in October 1968.

The total lipid content per mean individual worm (Fig. 3) showed considerable variation.

Increases in the lipid content of the worms in May and June coincided with similar increases in both the carbohydrate and protein components per worm. Similarly, the decrease in worm lipid content in late June and July, and the rise in September–October, matched similar changes for the other chemical constituents (Fig. 1, 2).

The data presented in Table 1 and Fig. 1–3 indicate the seasonal variation in the chemical constituents and food reserves at both the population and individual worm level. The relation between the size of individual worms and the size of the population is shown in Fig. 4.

The slope of the line clearly indicates the exponential decrease in average worm size as the number of individuals increases.

The relation of the chemical constituents and food reserves (weight per unit weight) to increasing population size is shown in Fig. 5.

This demonstrates that the *lyo*-glycogen fraction follows the relation between size of the individual worms and size of the population (see Fig. 4) and that *lyo*-glycogen is being utilised as population

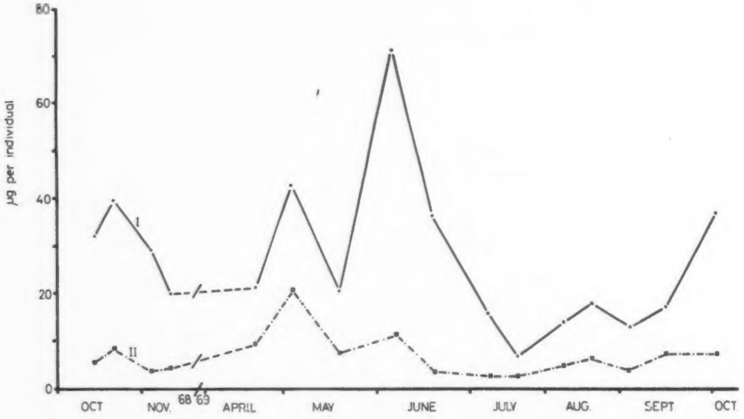

FIG. 1. Change in weight, expressed as µgrams per mean individual averaged from the sample, of (I) protein nitrogen and (II) nonprotein nitrogen, with time. Actual sample dates for each month are given in Table 1.

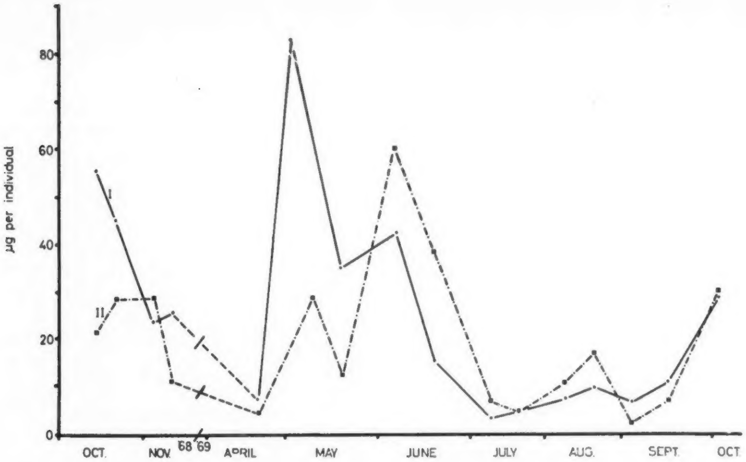

FIG. 2. Change in weight, expressed as µgrams per mean individual averaged from the sample, of (I) *lyo*-glycogen and (II) *desmo*-glycogen, with time. Actual sample dates for each month are given in Table 1.

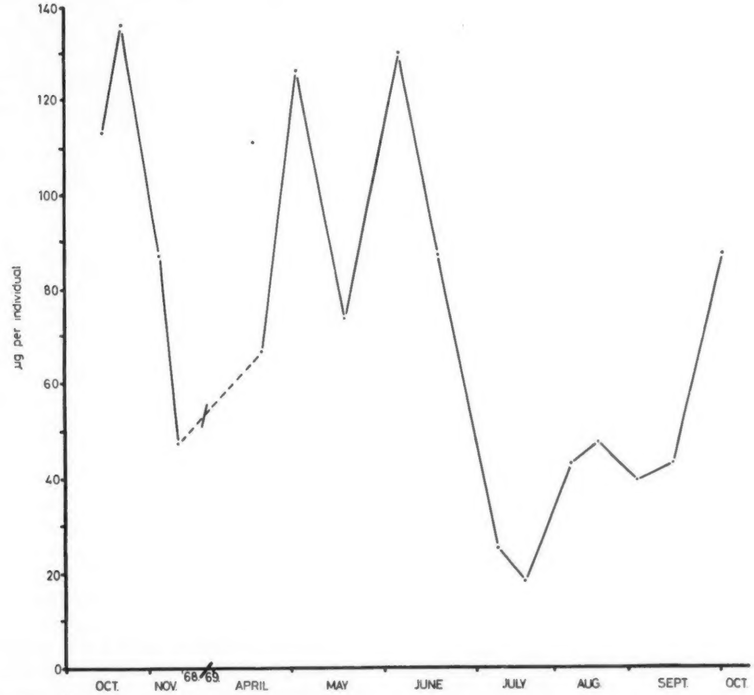

FIG. 3. Change in weight, expressed as µgrams per mean individual averaged from the sample, of total lipid, with time. Actual sample dates for each month are given in Table 1.

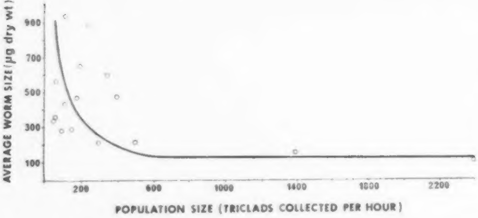

FIG. 4. Relation between average worm size (micrograms dry weight) and population size (individuals collected/hour).

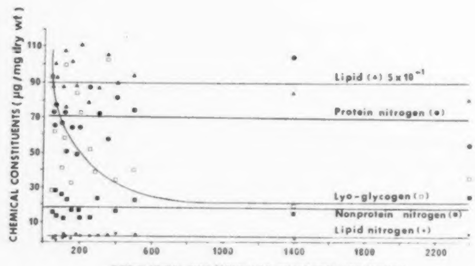

FIG. 5. Relation between chemical constituents (weight/unit weight) and population size (worms collected/hour).

size increases. In other food reserves, the amount present per unit worm weight remains constant.

Calculations of the regression coefficients of the chemical constituents and food reserves on the biomass of the population, derived from number of worms collected per hour × average worm size, confirms that there is a statistically significant ($P < 0.05$) decrease in the amount of *lyo*-glycogen present as biomass increases (see Table 2 and Fig. 6).

TABLE 2. Regression coefficient, standard error, and statistical significance of the regression lines of log chemical constituents against log biomass (avg worm size × no. of worms collected/hr) as shown in Fig. 6.

	Regression coefficient	SE	Statistical significance
Protein nitrogen	+0.008517	±0.05895	NS
Nonprotein nitrogen	−0.01763	±0.08424	NS
Lyo-glycogen	−0.17131	±0.1421	$P<0.05$
Desmo-glycogen	+0.09249	±0.1711	NS
Total lipid	−0.01804	±0.0342	NS

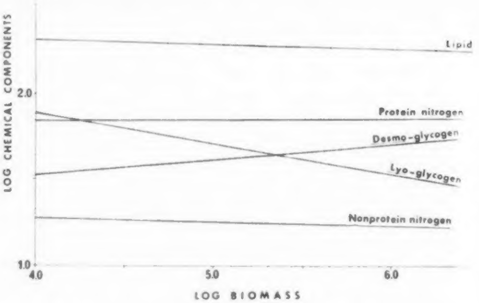

FIG. 6. Regression lines of log chemical constituents against log biomass (average worm size × number of worms collected/hour; see Table 1).

Discussion

The results detail the seasonal variation in population structure and in chemical constituents of *D. tigrina*. In evaluating the significance of the interaction between chemical constituents (including food reserves) and population structure, a number of other factors should be considered.

The only previous study on the population dynamics of *D. tigrina* was that of Armstrong (1964). The results of his laboratory experiments appear to have only limited application to field conditions.

One of the problems of a field study is the quantification of the size of the population. Under most conditions the timed hand-collection method suggested by Reynoldson (1966a) appears to be satisfactory. However, the method was considered to give a conservative estimate of the actual number of worms present when the number of worms found exceeded 400 per hour (Reynoldson 1966a). As is shown in Table 1, this number was surpassed on several occasions during the course of the present work.

The population decreased in numbers over October and November 1968 (see Table 1), so that by late November no flatworms were found in the littoral zone of the pond.

In mid-April 1969 a few flatworms were again found (≡60 collected per hour). The population size increased and reached a peak in July 1969, when as many as 400 worms could be collected in 10 min (≡2400 per hour). The population size began to decrease in August, and by mid-October no worms remained in the littoral zone of the pond.

Dugesia tigrina has a temperature preference of 13–23 C (Stokely et al. 1965). The disappearance of *D. tigrina* from the littoral zone in November 1968 and October 1969 may be directly correlated with the fall in water temperature below 13 C in these two months; similarly when the worms reappeared in April 1969 the water temperature was 14 C.

As *D. tigrina* follows a temperature gradient, and migrates to deeper waters when the temperature in the littoral zone is unfavourable, samples collected from the littoral zone at these times may not be representative of the total population.

In addition to variation in the size of the population, there is also considerable variation in the size of the individual members comprising the population (see Table 1). This latter variation can be accounted for by the life history of *D. tigrina*. The population of *D. tigrina* found in Grenadier pond reproduced in May and June. Laboratory experiments, not reported in this paper, showed that the worms were the genetic sexual strain (see Hyman 1941). In the field, fission was also seen to have occurred in May, June, and September. This confirms similar observations by Curtis (1902).

The occurrence of fission in the field population made it difficult to determine the time of peak hatching. Further, the relatively small number of cocoons found suggests that the major mode of population increase was by fission.

In May and June the population consisted of two generations, which explains the variability in dry weight per mean individual in these months (Table 1), and the changes per mean individual in nitrogen fractions (Fig. 1), carbohydrate fractions (Fig. 2), and lipid fraction (Fig. 3).

If changes within the triclad's population structure are controlled by intraspecific competition for a limited food resource, as has been suggested by Reynoldson (1966a) when the population size of *D. tigrina* started to increase in May–June, the ensuing competition would result in starvation, which, in turn, would affect the level of stored food reserves per worm.

Reynoldson (1966b) has shown in *Dugesia polychroa* (formerly *D. lugubris* — see Reynoldson and Bellamy 1970) and *Polycelis tenuis* that large adults were better able to survive under conditions of intraspecific competition than newly hatched young. This is probably because older worms could fall back upon their stored food reserves.

In the present study, the population reached its maximum size in July, which is when intraspecific competition for food would be at its greatest.

Under similar conditions of extended intraspecific competition, Reynoldson (1966b) has shown that young of *D. polychroa* and *P. tenuis* were better able to survive than adult individuals that, by tissue reabsorption, had shrunk to a comparable size. By inference it was therefore probably young individuals of *D. tigrina* that grew larger in August, as size of the population started to decline, in preference to shrunken individuals of the parent generation.

The results of the present investigation showed that at both individual and population levels there were changes in food reserves present. At the population level, the lipid, nitrogen, and *desmo*-glycogen fractions remained fairly constant over the year, indicating a constancy in food utilised in relation to number of worms present.

The small variation in protein and lipid fractions confirms observations of Willier et al. (1925), who showed that, in *Dugesia dorotocephala*, lipid and protein reserves showed little change after 12 weeks of starvation, and also those of Jennings (1957), who found lipids to be utilised slowly over an extended period of time.

The amount of *lyo*-glycogen decreased when population size increased (see Fig. 5), which implies that with an increase in number of individuals in the population there was a consequent utilisation of stored *lyo*-glycogen. This suggestion is supported by the statistically significant decrease in *lyo*-glycogen as population biomass increased (see Fig. 6).

Jennings (1957) has shown also that stored carbohydrates are the first food reserves to be utilised. From Table 1 it can be seen that the lowest values of *lyo*-glycogen were in late June and July, when the population size was at its greatest. The *lyo*-glycogen value was also low in April, which is in agreement with the results of von Brand (1936), who showed that *Planaria torva* used its carbohydrate food reserves in early spring.

At the individual level, there was a marked decrease in size of the worms as the population increased in numbers (see Fig. 4). This decrease in worm size was matched by decreases and changes in proportions of chemical components, with the exception of *lyo*-glycogen, present in individual worms.

After an initial rapid increase in early spring in the *lyo*-glycogen fraction per individual, there was an equally rapid decrease in this component coinciding with onset of the breeding period, and prior to the decrease in actual worm size. Apart from a slight rise in early June, the *lyo*-glycogen content per mean individual continued to fall until July. In contrast, the *desmo*-glycogen fraction per individual, although increasing at a slower rate in early spring, and showing a slight fall in late May, continued to increase until mid-June, before declining rapidly to a minimum value in late July. These changes indicate that in May and early June the *desmo*-glycogen component increased at the expense of the *lyo*-glycogen fraction, as confirmed by changes in the *desmo-:lyo*-glycogen ratios, although the amount and proportion of total carbohydrate present decreased. This suggests that during this period there was intraspecific competition for the food resource. After mid-June, intraspecific competition for the available food resource between both parent and offspring generations caused utilisation of stored carbohydrate reserves, and a marked decrease in worm size. When the number of individuals in the population decreased, worm size increased, again confirming that, with a relatively constant food resource, population structure was affected by intraspecific competition.

Maguire (1962, 1963) suggested that ectocrines control reproduction in *Cura foremanii*. In the present work on *D. tigrina*, the correlation between changes in chemical composition and changes in population structure is consistent with Reynoldson's (1966a) hypothesis that population size is regulated by food shortage. By supporting the implications of Reynoldson's hypothesis, these present results lend additional weight to acceptance of his hypothesis.

In a wider sphere the applicability of Reynoldson's (1966a) hypothesis to North American lake-dwelling triclads demonstrates that conclusions based on studies of European triclads may also be relevant to North American species, and should therefore be considered in future studies.

Acknowledgments

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Polymorphisms of Esterase Isozymes in the American Lobster (*Homarus americanus*)

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BARLOW, J., AND G. J. RIDGWAY. 1971. Polymorphisms of esterase isozymes in the American lobster (*Homarus americanus*). J. Fish. Res. Bd. Canada 28: 15–21.

Five groups of esterase active bands were found after electrophoretic separation of extracts of heart tissue of lobsters (*Homarus americanus*) on starch gels. Each group consisted of one or more bands that were distinguishable from other groups by their electrophoretic mobility substrate specificity, and susceptibility to various inhibitors. Groups designated I–IV are carboxylesterases; group V is a cholinesterase. Individual variations were observed in groups I, II, and V. The observed polymorphisms in groups I and II were hypothesized as due to codominant allelic systems. The phenotypic frequencies of group I isozymes observed in two populations agreed well with the Hardy–Weinberg distributions expected for such systems. The frequencies of variant types in group II were too low for meaningful population genetic analysis. Phenotype frequencies were not correlated with sex, size of the carapace, or stage of the molt cycle. Developmental changes of esterase isozymes were observed during the first eight stages. The Es-V (cholinesterase) group and Es-II groups were present at hatching, but the Es-I group was not clearly observed until after the seventh molt. Phenotype ratios of offspring from single females supported the genetic hypothesis for the Es-I system.

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GENETIC control of electrophoretic variants of esterases by codominant autosomal alleles has been demonstrated in a number of animals (see DeLigny 1968 for pertinent references). To find genetically controlled polymorphic systems of enzymes whose allelic frequencies are indicative of population structure in the lobster (*Homarus americanus* Milne Edwards), we have been analyzing the esterase isozymes of this species. This paper is a report on the electrophoretic and catalytic properties, possible genetic mechanisms, and frequency distribution of phenotypes of esterases in lobsters.

Materials and Methods

Lobsters used in this study were obtained from several sources. Those from coastal areas were purchased from local dealers or directly from fishermen; a few were obtained by scuba diving. Lobster samples from offshore areas were taken by trawling during research cruises of the RV *Albatross IV* and the RV *Delaware*.

Stages of the molt cycle were designated as previously described (Barlow and Ridgway 1969). Larval and juvenile stages were classified by the number of molts, stage I being the newly hatched larvae and stage II after the first molt, etc. Stages were identified according to descriptions of Herrick (1911). Beginning with stage IV the small lobsters were considered as juveniles rather than larval lobsters. The stage IV lobster has almost all of the principal characters of the adult and is no longer free-swimming.

Larval and juvenile lobsters were obtained from egg-bearing females caught in offshore waters on a research cruise of the RV *Delaware* in October 1968. The females were kept in the laboratory tanks at ambient seawater temperature until November and then put in a temperature-controlled tank at 22 C. They had extruded their eggs about September 1968, and began to hatch larvae in mid-January 1969, about 4 months before lobsters in the natural environment. The larvae from each female were kept in round glass tanks 22 cm in diameter and 29 cm deep. The tanks were fitted with a recirculating system that included an ultraviolet sterilizer and a charcoal and glass-wool filter. To prevent cannibalism, vigorous circulation of the larvae was accomplished by active aeration. Larvae were fed a constant supply of newly hatched brine shrimp (*Artemia salina*). Juveniles of stage IV and older were each kept in an individual petri dish at room temperature and fed small pieces of clam muscle or mantle once each day. The water was changed about 2 hr after feeding; all excess food was removed when the water was changed. Stage VIII juveniles were obtained in about 2 months by these procedures.

Tissues to be analyzed for isozymes were removed and either stored in liquid nitrogen or homogenized immediately (frozen hearts were kept from 1 to 2 months in liquid nitrogen and homogenized while still frozen) with a mortar and pestle in an equal amount of water

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(w/v). The homogenate was centrifuged at 14,000 *g* for 30 min at 5 C and the supernatant used for electrophoresis. Isozymes were extracted from juvenile and larval lobsters by grinding the entire animal in an equal amount of water.

Horizontal starch-gel electrophoresis (Smithies 1955) was carried out using a *p*H 8.65 electrode buffer (20 g tris (hydroxymethyl) amino methane (Tris), 2 g disodium EDTA, and 3.8 g boric acid per liter). Gel buffer was prepared by reducing the *p*H of the electrode buffer to 8.20 with 1 N HCl and then diluting to three-tenths the original concentration. Thirty μliters of the supernatant were applied to a 4 × 6-mm piece of Beckman #31929 paper wick and inserted into a slit cut 5.5 cm from the cathode end of the gel. Direct current was applied for 30 min at 7 v/cm, after which the sample blotters were removed and the gel run an additional 240 min at 15 v/cm.

After completion of electrophoresis the gels were sliced horizontally with a continuous band razor blade and incubated in a solution of 92 ml water, 4 ml phosphate buffer (0.4 N, *p*H 6.55), 4 ml 1% substrate in acetone or alcohol and 0.1 g of fast blue RR. Substrates utilized were α-naphthyl acetate; β-naphthyl acetate; naphthol ASD acetate; α-naphthyl butyrate; β-carbonaphthoxy choline iodide; 6-bromo-2-carbonaphthoxy choline iodide; α-naphthyl laurate; β-naphthyl laurate; β-naphthyl myristate; β-naphthyl proprionate; and β-naphthyl stearate. Gels were stained for 30 min–24 hr (depending upon the substrate used) and then decolorized and stored in 30% ethanol.

Inhibitor studies were performed by incubating half of the cut gel in a buffered inhibitor solution for 30 min prior to esterase staining. The staining solution also contained the inhibitor in the same concentration. Chemical inhibitors tested were BW 284-C-51; BW 62-C-47 ("specific" and "generalized" anticholinesterases from Burroughs Wellcome & Co.)[2]; *p*-chloromercurobenzoic acid; eserine salicylate and eserine sulfate; N-ethyl maleimide; iodoacetamide; iodoacetate; sodium lauroyl sarcosine; sevin[2] (1-naphthyl N-methylcarbamate); and dichlorvos (vapona[2], Shell Chemical Co., 2,2-dichlorovinyl dimethyl phosphate). Controls were run by incubating half of the gel in the reaction mixture without the inhibitor. The effects of temperature and *p*H changes were tested with the same procedures, with one-half of the gel incubated at room temperature and *p*H 6.55.

Single isozyme bands were isolated for a rerun with starch-gel electrophoresis by staining the top half of the gel and cutting out the band from the bottom half. The strip of gel containing the isolated band was then used in place of the sample blotter in a second gel and rerun.

Results and Discussion

PRELIMINARY SURVEY OF ESTERASES

With α-naphthyl acetate and α-naphthyl butyrate as substrates a survey of esterase enzymes was made in the following tissues of the lobster: serum, heart, eye and stalk, green gland, hepatopancreas, ovary, intestine, claw muscle, abdominal flexor muscle, and abdominal extensor muscle. The electrophoretic pattern of esterase bands was similar in most of the tissues examined but the intensity of staining and number of bands visible appeared most complex in the eye and stalk, hepatopancreas, intestine, and heart (Fig. 1). The ovary, hepatopancreas, and the two abdominal muscles show bands that are not present in other tissues; however, most of the bands are present in all the tissues except serum. Only one band of esterase was present in the serum. The heart tissue was chosen for analysis of polymorphic forms for the following reasons: (1) the bands of esterase activity were more readily observed in the heart than any other tissue; (2) the heart tissue was less likely to reflect the physiological variation expressed in other tissues during the molt and reproductive cycles (Barlow and Ridgway 1969); and (3) the heart could easily be removed and preserved in liquid nitrogen under field conditions.

ELECTROPHORETIC AND CATALYTIC PROPERTIES OF HEART ESTERASES

As many as 14 different bands of esterase activity were observed after electrophoretic separation of lobster heart extracts in starch gel. These bands were divided into five groups according to their substrate and inhibitor specificities and their position on the starch-gel zymogram (Fig. 2).

Tables 1 and 2 summarize the substrate and inhibitor specificity of esterases according to group. The effects of various *p*H values from 5.0 to 7.5 were studied, but all zones stained the same, with maximum activity at *p*H 6.55. The group V bands are classified as cholinesterases because of their sensitivity to low concentrations of eserine; groups I–IV are classified as carboxylesterases (formerly called aliesterases) as described by Augustinsson (1961). These esterases have a wide specificity, are inhibited by organophosphorus compounds, act on simple esters of low molecular weight acids, and are not inhibited by 10^{-5} M eserine, by 10^{-3} M *p*-chloromercurobenzoate, nor by EDTA. Since the gel buffer and electrode buffer contained EDTA in 5×10^{-4} and 5×10^{-3} M concentrations, the esterases are not inhibited by these concentrations

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[2] Trade names referred to in this publication do not imply endorsement of commercial products. We are grateful to the Burroughs Wellcome & Co., Inc. for supplying us with their compounds 284-C-51 and 62-C-47 and to Shell Chemical Co. for supplying us with a sample of vapona.

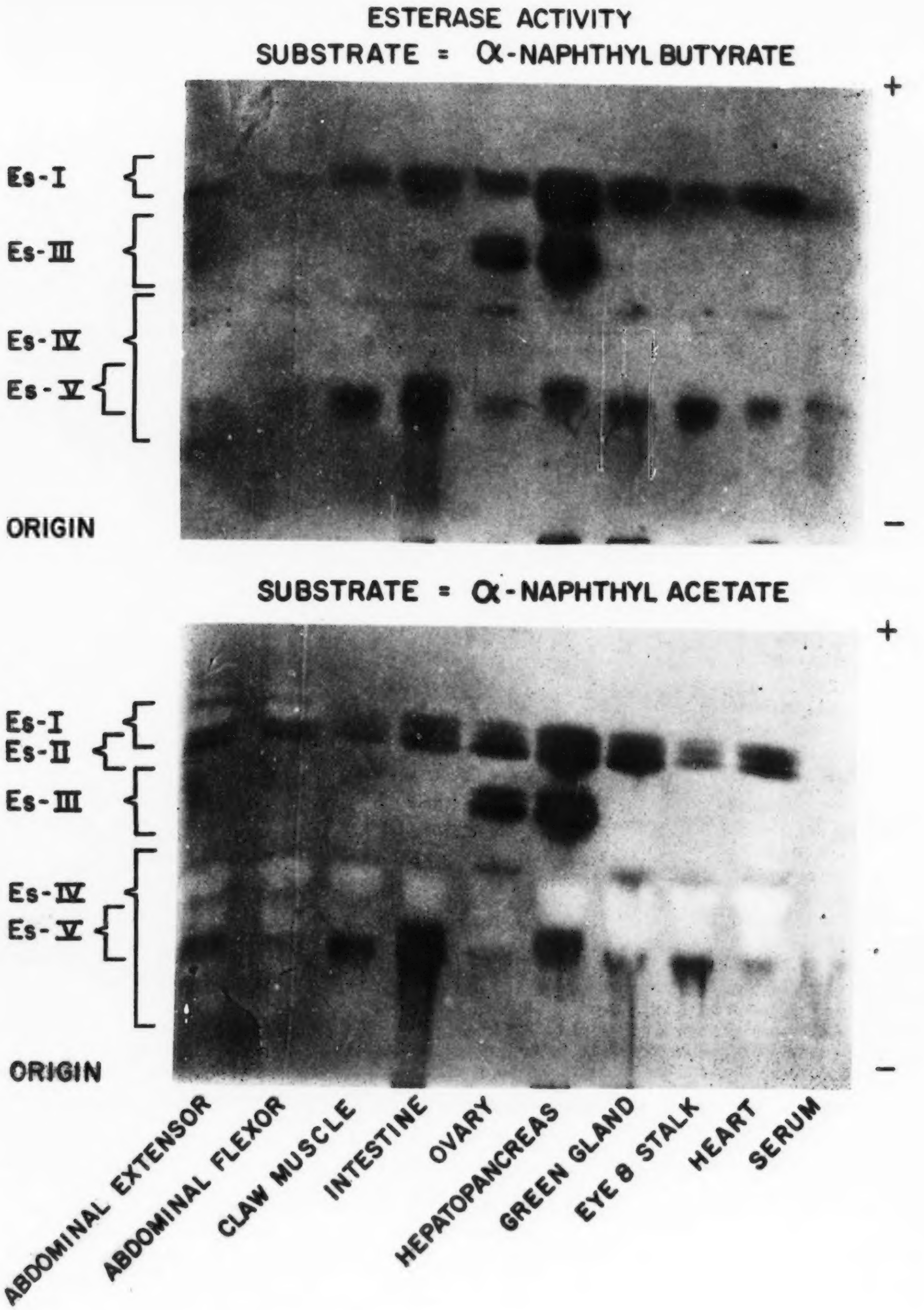
ESTERASE ACTIVITY
SUBSTRATE = α-NAPHTHYL BUTYRATE
+
Es-I
Es-III
Es-IV
Es-V
ORIGIN
−
SUBSTRATE = α-NAPHTHYL ACETATE
+
Es-I
Es-II
Es-III
Es-IV
Es-V
ORIGIN
−
ABDOMINAL EXTENSOR
ABDOMINAL FLEXOR
CLAW MUSCLE
INTESTINE
OVARY
HEPATOPANCREAS
GREEN GLAND
EYE & STALK
HEART
SERUM

FIG. 1. Esterases in various tissues of a single female lobster. Both zymograms are from the same gel but stained with different substrates. The groups of bands marked on the side of the figure refer to heart isozyme groups (350 v for 5 hr, horizontal).

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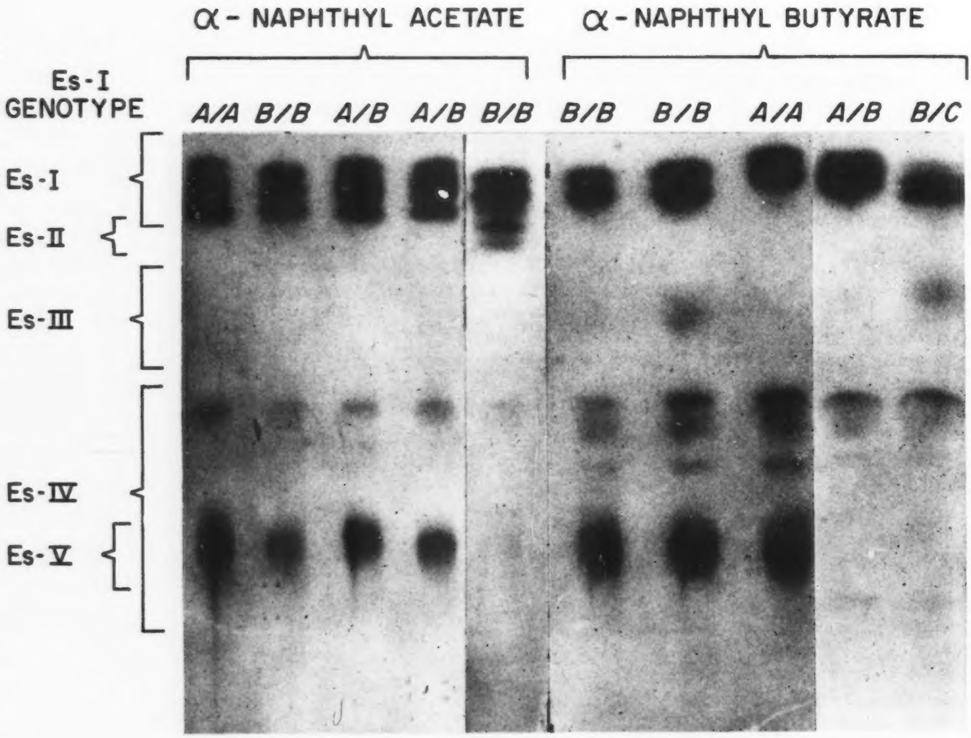
α - NAPHTHYL ACETATE
α - NAPHTHYL BUTYRATE
Es-I
GENOTYPE
A/A
B/B
A/B
A/B
B/B
B/B
B/B
A/A
A/B
B/C
Es-I
Es-II
Es-III
Es-IV
Es-V

FIG. 2. Composite of esterase zymograms showing various zones of activity and the Es-I and Es-II phenotypes. In the fifth sample the Es-II genotype is A/B, the Es-II genotypes of all other samples are A/A. The fifth, ninth, and tenth samples were stained in a solution that contained eserine sulfate (10^{-4} M); note that zone V is inhibited.

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TABLE 1. Comparison of the staining intensity of zones of esterase activity of lobster heart protein by various substrates.

Substrate	Staining intensity[a] of zones				
	I	II	III	IV	V
α-naphthyl acetate	+++	+++	++	++	+++
β-naphthyl acetate	+++	+++	++	++	+++
Naphthol ASD acetate	–	–	–	–	+
α-naphthyl butyrate	+++	–	+++	+++	+++
β-carbonaphthoxy choline iodide	–	–	–	–	–
6-bromo-2 carbonaphthoxy choline iodide	–	–	–	–	++
α-naphthyl laurate	–	–	–	–	–
β-naphthyl laurate	–	–	–	–	+
β-naphthyl myristate	+	–	–	–	–
β-naphthyl proprionate	++	–	–	++	+++
β-naphthyl stearate	–	–	–	+	–
α-naphthyl acetate plus β-naphthyl acetate[b]	Blue	Blue	Blue	Blue	Red

[a] + (slight staining); ++ (medium staining); +++ (dark staining); – (no staining).

[b] α-naphthyl acetate stains blue; β-naphthyl acetate stains red.

of EDTA. Most of the substrates used for characterizing carboxyl esterase activity may also be hydrolyzed by proteolytic enzymes (such as trypsin and chymotrypsin). Proteases, however, are not highly sensitive to organophosphorus compounds such as dichlorvos (Clements 1967). The esterase groups (I–V) probably represent discrete genetic systems since the inhibitor and substrate sensitivity are equal in all bands of a single group and differ among groups.

Group I is the fastest migrating of the esterase groups and consists of two, three, or four visible zones of activity. The slower band of group I overlaps the faster band of group II. This band was distinguished from the group II band by its ability to hydrolyze α-naphthyl butyrate.

Group II bands differ from group I by not hydrolyzing α-naphthyl butyrate and not being inhibited by 10^{-3} M sevin. Group II bands are also more stable to aging and freezing than group I and exhibit only one polymorphic variant.

The group III bands of esterase activity appear about midway on the zymogram. These bands are distinguished primarily by location but they also differ slightly in substrate and inhibitor activities from the other groups (Table 1, 2). No variation other than intensity of staining has been noted in this group and very prolonged staining is necessary to obtain a clear set of bands.

Group IV comprises five esterase bands that differ in electrophoretic mobility and intensity of staining. Two of these bands are visible only when group V (cholinesterase) has been inhibited. The faster migrating bands in group IV appear darker and the slower bands are progessively lighter. This suggests that some type of polymerization of active subunits is producing this pattern. No variation in phenotype has been noted in this group.

Group V esterase occurs as a single band identified as cholinesterase by its sensitivity to eserine sulfate. This band may appear in fast, intermediate, or slow forms. The diffuseness of the bands hampers identification. In several zymograms the cholinesterase bands differed in mobility in different tissues of the same lobster (Fig. 1). Maynard (1964) found two forms of cholinesterase in *H. americanus* nervous tissue, and it is probable that the variation observed between tissues of a single lobster is due to the quantitative variation in different tissues of at least two cholinesterase forms.

POLYMORPHISM AND GENETICS

Group I esterases were found in three common phenotypes and two less frequently observed phenotypes (Fig. 2, 3). The two group I bands exist together in fast or slow forms or in both the fast and slow forms. These three phenotypes termed Es-I-A/A, Es-I-A/B, and Es-I-B/B are considered to be the result of codominant allelic inheritance at a single autosomal locus (Es-I^{A} = fast allele, Es-I^{B} = slow allele). Each allele controls two isozyme

TABLE 2. Interzonal comparison of the inhibition of bands of esterase activity of lobster heart protein by various inhibitors.

			Inhibition of zones[b]				
Inhibitor and concentration		Substrate[a]	I	II	III	IV	V
BW[c] 284-C-51	10^{-3} M	NA	–	–	–	–	+
	10^{-3}	NB	–	0	–	–	+
BW[c] 62-C-47	10^{-3}	NA	–	–	–	–	–
	10^{-3}	NB	–	0	–	–	–
p-chloromercurobenzoic acid	10^{-2}	NA	–	–	–	–	–
	10^{-2}	NB	–	0	–	–	–
Eserine salicylate	10^{-3}	NA	+	–	?	–	+++
	10^{-5}	NA	+	–	–	–	+++
	10^{-3}	NB	+	0	+	–	+++
	10^{-5}	NB	+	0	–	–	+++
Eserine sulfate	10^{-3}	NA	++	–	+++	–	+++
	10^{-6}	NA	+	–	+	?	+++
	10^{-3}	NB	++	0	+	–	+++
	10^{-6}	NB	–	0	+	–	+++
N-ethyl maleimide	10^{-3}	NA	–	–	–	–	–
	10^{-3}	NB	–	0	–	–	–
Iodoacetamide	10^{-3}	NA	–	–	–	–	–
	10^{-3}	NB	–	0	–	–	–
Iodoacetate	10^{-3}	NA	–	–	?	–	–
	10^{-3}	NB	–	0	+	–	–
Sodium lauroyl sarcosine	10^{-3}	NA	–	–	–	–	–
	10^{-3}	NB	–	0	–	–	–
Sevin	10^{-3}	NA	++	–	?	–	+++
	10^{-3}	NB	+++	0	+	–	+++
Dichlorvos	10^{-3}	NA	+++	+++	+++	+++	+++
	10^{-5}	NA	+++	+++	+++	+	+++
	10^{-6}	NA	+	++	?	?	–
	10^{-3}	NB	+++	0	+++	+++	+++
	10^{-5}	NB	+++	0	+++	–	+++
	10^{-6}	NB	+	0	?	–	–
65 C for 20 min		NA	+++	+++	+++	+++	+++
		NB	++	0	+++	–	+++

[a] NA = α-naphthyl acetate; NB = α-naphthyl butyrate.

[b] + (slight inhibition); ++ (moderate inhibition); +++ (complete inhibition); — (no inhibition); ? (not clear); 0 (does not react with substrate).

[c] BW = Burroughs Wellcome & Co., Inc. compound.

bands that overlap in the heterozygote Es-I^{A}Es-I^B. This was tested by mixing equal amounts of extract from Es-I-A/A and Es-I-B/B individuals; the resulting pattern was that of Es-I-A/B. The two rarer phenotypes Es-I-A/C and Es-I-B/C are considered to be the result of a third allele at the same locus (Es-I^C = very slow allele). The hypothesized Es-I^{C}Es-I^C homozygote was not observed.

Multiple esterase bands controlled by a single allele have been noted by a number of authors (Allen 1961; van Asperen 1964; DeLigny 1968; Gahne 1966; Grunder et al. 1965; Nyman 1967; Popp and Popp 1962; Tashian and Shaw 1962). Several hypotheses may be advanced to explain

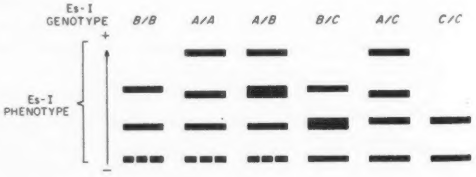

FIG. 3. Electrophoretic patterns of the five observed Es-I phenotypes and one hypothetical phenotype (C/C). The broken bands indicate the fast band of the Es-II system that is distinguishable from the slow C band of the Es-I system by its inability to hydrolyze α-naphthyl butyrate.

the existence of two bands controlled by a single allele. The interaction of macromolecules and buffer may produce two stable zones from a single macromolecular species (Cann and Goad 1965, 1968). Conformational isomers from a single molecular species might also give two bands after electrophoresis. To test the first of these hypotheses each band was isolated from extracts of Es-I-B/B and Es-I-A/A phenotypes and subjected to a second run in a second starch-gel zymogram. These bands did not split into two bands as would be expected if the molecules were interacting with buffer components. This test, however, did not eliminate the possibility of conformational isomers. To test this possibility each band would have to be isolated and then subjected to reversible denaturation such as freeze and thaw, rerun on a second gel, and observed to see if two bands were formed from a single band. It was not possible to isolate sufficient amounts of either band to carry out this experiment. A third hypothesis is that multiple isozymes may be formed from a single active subunit that dimerized with itself to produce a second band. The expected position of such bands on the zymogram does not coincide with that observed. A fourth hypothesis is that each isozyme is a complex protein molecule of at least two subunits, with each band having one common subunit and differing in at least one other subunit. This hypothesis requires a second genetic locus with no polymorphism.

Observed and expected phenotypic frequencies for the Es-I system from several different geographic areas were compared to test the genetic hypothesis. The observed frequencies of phenotypes were all in close agreement with those expected from the Hardy–Weinberg distribution (Table 3).

The two phenotypes that have been observed for group II esterase are a single band and three evenly spaced bands (Fig. 2). By assuming a dimeric enzyme (2 subunits) coded for by a single locus with codominant alleles (Es-II^{A} and Es-II^{B}) the single fast band would be a homozygote Es-II-A/A phenotype; the triple-banded phenotype would be the result of random assortment of subunits in the heterozygote (Es-II-A/B). The other hypothesized homozygote would exist in very low frequency and has not yet been observed. The staining intensity of the three bands seems to indicate a 1:2:1 distribution (Fig. 2), which would be expected if this hypothesis were true. Similar inheritance of esterases with hybrid enzymes formed in the heterozygote have been found in many animals (see Shaw 1965 for examples).

Because of a low allelic frequency for the Es-II^{B} gene (0.03) no population data could be used to test the Es-II genetic hypothesis.

No polymorphism was seen in group III and IV esterases. Variation of the group V esterases was seen in different tissues of the same lobster, and no genetic analysis was possible.

As a further check on whether the polymorphisms observed in esterase group I and II were genetic or possibly related to some physiological variation, observations were kept on the sex, size, and molt cycle stage of each sample. Contingency tables were constructed and a chi-square test run. No significant variation in the expected frequencies of Es-I or Es-II phenotypes was found within the various groups (sex, size, and molt stage). This contrasts with the variations observed in serum proteins during the molt and reproductive cycles (Barlow and Ridgway 1969).

TABLE 3. Comparison of observed phenotypic frequencies of lobster heart esterases with expected frequencies in samples from two locations.

	Phenotypes Es-I						Chi-square	Probability
	B/B	B/A	A/A	B/C	A/C	C/C		
	Inshore (Boothbay Harbor, Maine)							
Observed	82	79	13	6	1	0	1.13	50–75
Expected	85.2	72.4	15.4	4.7	1.8	0		
	Offshore[a]							
Observed	63	61	8	3	5	0	1.90	25–50
Expected	64.3	55.6	12.0	5.3	2.3	0		

[a] Offshore samples were taken along the edge of the continental shelf between Hudson and Lydonia canyons in depths of 20–about 200 fath.

OFFSPRING PHENOTYPES

Offspring were reared from five females; larvae and juveniles were sampled at each stage of development and zymograms prepared for esterases using α-naphthyl acetate and α-naphthyl butyrate as substrates. The whole animal was homogenized rather than just heart tissue; the identity of the larval esterase groups was confirmed by using eserine sulfate and sevin (10^{-3} M) inhibition patterns. The Es-V (cholinesterase) group and Es-II groups were easily identified at stage I, but the Es-I group did not become clearly visible until stage VIII.

The number of different offspring phenotypes for the Es-I isozyme system and the phenotype of the female parent are given in Table 4. Only two juveniles were available from female number III and only six from female number IV. It was not possible to establish a ratio of offspring phenotypes from these low numbers, but all of the juvenile phenotypes observed fit the proposed genetic hypothesis. Lack of variation in the Es-II system prevented any testing of the genetic mechanism involved with this system. The phenotype of the male parent (Table 4) was obtained from the ratio of offspring, assuming the genetic hypothesis is correct.

The three females that provided sufficient juveniles to obtain a good estimate of the phenotypic ratios in the offspring for the Es-I system were all A/B heterozygotes. The expected ratios for a heterozygote are 1:1 if mated with a homozygote (A/B × B/B = 50% A/B:50% B/B and A/B × A/A = 50% A/B:50% A/A) and 1:2:1 if mated with another heterozygote (A/B × A/B = 25% A/A:50% A/B:25% B/B). Not all these ratios were observed, but those observed fit the genetic hypothesis. No C alleles were involved in the animals examined.

SIGNIFICANCE OF RESULTS

Genetic hypotheses have been suggested for two heart esterase systems. Evidence for the method of inheritance of the Es-I phenotypes was obtained from population genetics and from the offspring ratios of single female lobsters. According to this evidence the Es-I system is controlled by three codominant alleles at a single locus; each allele controls two bands that overlap in the heterozygous condition. Direct evidence for the method of inheritance of the Es-II phenotypes is not available. The Es-II system is hypothesized to be inherited by two codominant alleles at a single locus with three phenotypes. An attempt to test this hypothesis through progeny analysis was unsuccessful, since no variation was observed. The low frequency of the Es-II allele in the wild samples likewise precluded meaningful analysis. There was no evidence that these enzymes were affected by sex, size, or stage of the molt cycle.

The objective of this study was to find an esterase polymorphic system that would allow comparisons of allelic frequencies in lobster stocks from different geographic areas. The esterase histochemical method resulted in the staining of a number of diverse nonspecific esterase systems, but when coupled with inhibitor and activator studies it was possible to distinguish single enzyme systems. Codominant allelic variation expressed in such systems has been seen in many species and enzyme systems (Shaw 1969). The esterase isozymes are readily demonstrated by the starch-gel electrophoretic techniques and exist in polymorphic forms that allow determination of allelic frequencies directly from the phenotypic frequencies. These enzyme systems should thus be of value in comparing gene frequencies in various local populations.

Studies on enzyme polymorphisms have indicated that a large amount of genetic heterozygosity exists in natural populations (Gillespie and Kojima 1968; Hubby and Lewontin 1966; Lewontin and Hubby 1966; MacIntyre and Wright 1966; O'Brien and MacIntyre 1969). Natural populations of lobsters are dispersed over a large geographic area but might maintain gene flow by larval drift and direct migration. Variation in gene frequency between populations from different areas should

TABLE 4. Es-I phenotypes of offspring from individual female lobsters.

Female parent		Offspring no. for Es-I phenotypes						Offspring ratio	Possible phenotype of male parent
No.	Phenotype	B/B	A/B	A/A	B/C	A/C	C/C		
I	A/B	15	13	0	0	0	0	1:1	B/B
II	A/B	0	13	13	0	0	0	1:1	A/A
III	A/B	1	1	0	0	0	0	?	B/B or A/B
IV	B/B	6	0	0	0	0	0	0:1	B/B
V	A/B	27	21	0	0	0	0	1:1	B/B

be indicative of the degree of genetic isolation. Uniform gene frequencies over wide geographic areas will indicate either gene flow maintained by migration and larval transport, or perhaps uniform selective forces over the entire area. We are currently examining the frequency distributions of the esterase systems reported here and those of several other enzyme systems.

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Taxonomic Status of *Calanus finmarchicus* and *C. glacialis* (Copepoda), with Special Reference to Adult Males[1]

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Frost, B. W. 1971. Taxonomic status of *Calanus finmarchicus* and *C. glacialis* (Copepoda), with special reference to adult males. J. Fish. Res. Bd. Canada 28: 23–30.

A morphometric analysis using certain taxonomic characters of adult males of *Calanus finmarchicus* (Gunnerus) and *C. glacialis* Jaschnov shows that the taxa comprise two nonoverlapping clusters. *Calanus finmarchicus* and *C. glacialis* are probably reproductively isolated. Differences between the species in geographical distribution, vertical distribution, and timing of life cycles also support this conclusion. Previous taxonomic studies of adult females of the two species have yielded inconclusive results, probably because the analyses were based largely on subgeneric characters.

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In the temperate North Atlantic Ocean and Arctic Ocean, representatives of the copepod genus *Calanus* are often the most prominent components of the marine zooplankton. Ecological studies of this genus are hampered because of uncertainties about the taxonomic status of certain species. The North Atlantic and Arctic species comprise two groups: a single distinctive species, *C. hyperboreus* (Krøyer), and a group of three morphologically similar taxa that have been named *C. finmarchicus* (Gunnerus), *C. glacialis* Jaschnov, and *C. helgolandicus* (Claus).

Currently there are three views concerning the taxonomic relationships of the latter three taxa. Jaschnov (1955, 1957) and Brodsky (1959, 1967a, b) consider all three to be valid species. Matthews (1967) recognizes the species *C. finmarchicus* and *C. helgolandicus*, but thinks that *C. glacialis* is a subspecies of *C. finmarchicus*. Aurich (1966) concludes that all three taxa are geographical races of *C. finmarchicus*. The confusion about these three groups of *Calanus* is due to the apparent lack of distinctive morphological differences among them, particularly in comparison with groups of species in many other genera of calanoid copepods. For example, *C. finmarchicus* and *C. glacialis* were originally distinguished by Jaschnov (1955) primarily on the basis of adult size and structure of the male and female fifth swimming legs. The two taxa appear to intergrade slightly in all of the characters used by Jaschnov (Grainger 1961; Aurich 1966; Matthews 1966, 1967).

Judged from patterns of morphological divergence in other species of *Calanus* (Brodsky 1967b), the fifth swimming leg of the adult male might be expected to show some divergence if indeed populations of *C. finmarchicus* and *C. glacialis* are reproductively isolated. The purpose of this paper is to present an interpretation of the taxonomic status of *C. finmarchicus* and *C. glacialis*, based on a refinement and morphometric analysis of some characters that Jaschnov (1955) used to distinguish adult males.

Materials and Methods

The morphometric analysis is based on a collection of adult males fitting the descriptions of *Calanus finmarchicus* and *C. glacialis* from samples scattered throughout the North Atlantic and Arctic oceans. Samples (Table 1) were available from areas where *C. finmarchicus* and *C. glacialis* are allopatric (Norwegian Sea and central Arctic Ocean) as well as from areas of sympatry (Barents Sea and Greenland Sea). Of 349 adult males examined, 133 were selected for measurement. To investigate allometric effects, specimens were chosen from samples to provide the widest possible range of size. However, within several series two or more specimens of nearly the same size were measured. Morphological terminology follows that of Gooding (1957) and Fleminger (1967). Measurements were made on specimens that had been immersed in a 50:50 solution of glycerine and sea water. The prosome was measured in lateral view from the anterior margin of the head to the posterior margin of the last thoracic segment. Measurements were taken at 25× with an ocular micrometer read to the nearest whole micrometer unit (micrometer unit = 0.039 mm). A factor of about 1.3 converts prosome length to total length.

Only length measurements of individual segments of the left fifth swimming leg of the male are reported. The entire leg was mounted anterior side upward on a glass slide and gently flattened under a glass cover slip.

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[1] Contribution No. 558 from the Department of Oceanography, University of Washington, Seattle, Wash.

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TABLE 1. Number and collecting locality of adult males of *Calanus finmarchicus* and *C. glacialis* used in the morphometric analysis. Samples were collected from ice island *Arlis II* (Greenland Sea), ice island T-3 (central Arctic Ocean), the USCGS *Eastwind* (Barents Sea), and vessels of the Norwegian Fishery Research Institute (expedition "Polarfront" in the Norwegian Sea).

Location		Date of collection	*C. finmarchicus*	*C. glacialis*
		Greenland Sea		
75°00′N	09°53′W	Feb. 1965	3	24
77 01	11 05	Mar. 1965	5	2
76 56	10 54	" "	4	7
74 10	14 53	" "	1	–
75 48	15 38	" "	10	9
69 45	19 20	Apr. 1965	7	7
		Barents Sea		
77 28	75 20	Aug. 67	6	5
77 21	59 20	" "	–	2
78 40	71 22	" "	1	2
80 26	72 36	" "	–	1
		Norwegian Sea		
66 00	02 00	Mar. 1952– July 1952	30	–
		Central Arctic Ocean		
71 35	150 10	Feb. 1960	–	1
75 29	155 18	Mar. 1966	–	1
84 48	127 12	Apr. 1969	–	4
84 46	127 23	Apr. 1969	–	1

Measurements of segment lengths were made along a line parallel to the long axis of the segments. The limits of length measurements of exopodal segments were taken as follows: the first segment (Fig. 1A, Re_1), from the proximal anterior edge, defined by a small condyle on the second basipodal segment (Fig. 1B), to the distal medial edge; the second segment (Fig. 1A, Re_2), from the proximal medial edge to the distal anterior margin just medial to the distal spinous process; the third segment (Fig. 1A, Re_3), from the proximal lateral edge to the distal medial edge at the base of the long distal seta. The length of the second endopodal segment (Fig. 1C, Ri_2) was measured from the proximal margin, defined by a small condyle on the first endopodal segment (Fig. 1B), to the distal anterior margin. The limits of these measurements were arbitrarily chosen; they were easily seen on all specimens examined. A few length measurements, not reported here, were made of the first and third endopodal segments (Fig. 1C, Ri_1, and Ri_3).

Segments of the fifth swimming legs on males equal to or larger than 3.2-mm prosome length were measured at 100× with an ocular micrometer read to the nearest whole micrometer unit (micrometer unit = 0.005 mm). Legs on males smaller than 3.2-mm prosome length were measured at 400× with an ocular micrometer

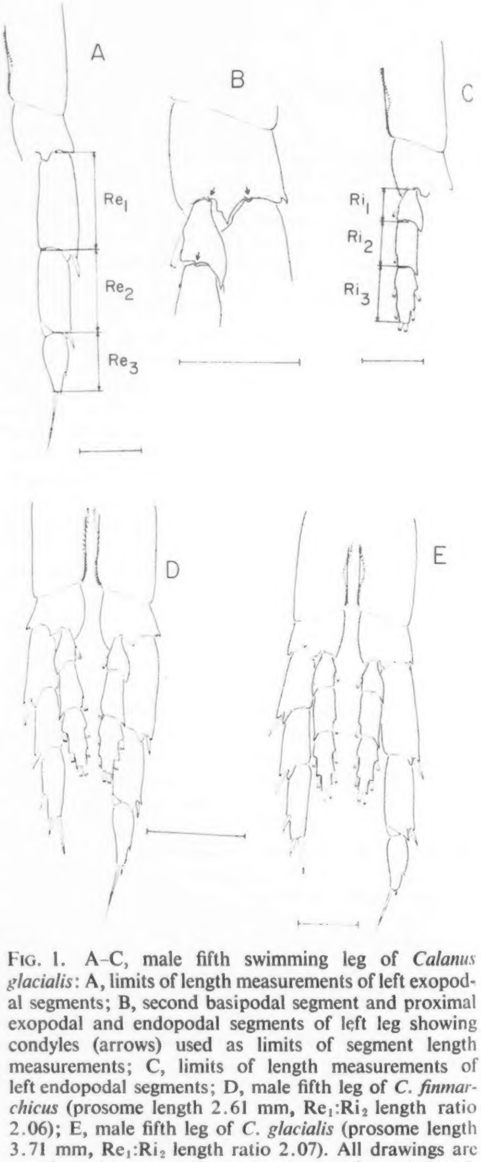

FIG. 1. A–C, male fifth swimming leg of *Calanus glacialis*: A, limits of length measurements of left exopodal segments; B, second basipodal segment and proximal exopodal and endopodal segments of left leg showing condyles (arrows) used as limits of segment length measurements; C, limits of length measurements of left endopodal segments; D, male fifth leg of *C. finmarchicus* (prosome length 2.61 mm, $\mathrm{Re}_1{:}\mathrm{Ri}_2$ length ratio 2.06); E, male fifth leg of *C. glacialis* (prosome length 3.71 mm, $\mathrm{Re}_1{:}\mathrm{Ri}_2$ length ratio 2.07). All drawings are anterior views; complete armature and total length of all setae not shown. Scales are 0.2 mm.

(micrometer unit = 0.001 mm). All males larger than 3.2 mm in prosome length had fifth leg segments (Re_1) that were longer than the range of the ocular micrometer when used at 400×.

Results

Measurements of prosome length of the 133 adult males ranged from 2.34 to 4.10 mm (total length about 3.04–5.33 mm). Comparison of proportional lengths of the three exopodal segments showed that only the lengths of Re_1 and Re_2 vary discontinuously with prosome length. Length of the Re_3 and lengths of all three endopodal segments vary by a constant proportion with prosome length throughout the range of prosome measurements taken.

When plotted against prosome length, measurements of Re_1 fall along two regression lines with statistically identical slopes but different mean Re_1 lengths (Fig. 2). A sharp shift in Re_1 measurements occurs in the scatter diagram between 3.16- and 3.20-mm prosome length. Similar results are apparent for the scatter diagram of prosome length and Re_2 length (Fig. 3). In Fig. 2 and 3 the specimens equal to or smaller than 3.16-mm prosome length are identified as *Calanus finmarchicus*; the larger specimens are *C. glacialis*. When Re_3 lengths of *C. finmarchicus* and *C. glacialis* are plotted against prosome length (Fig. 4), the points are

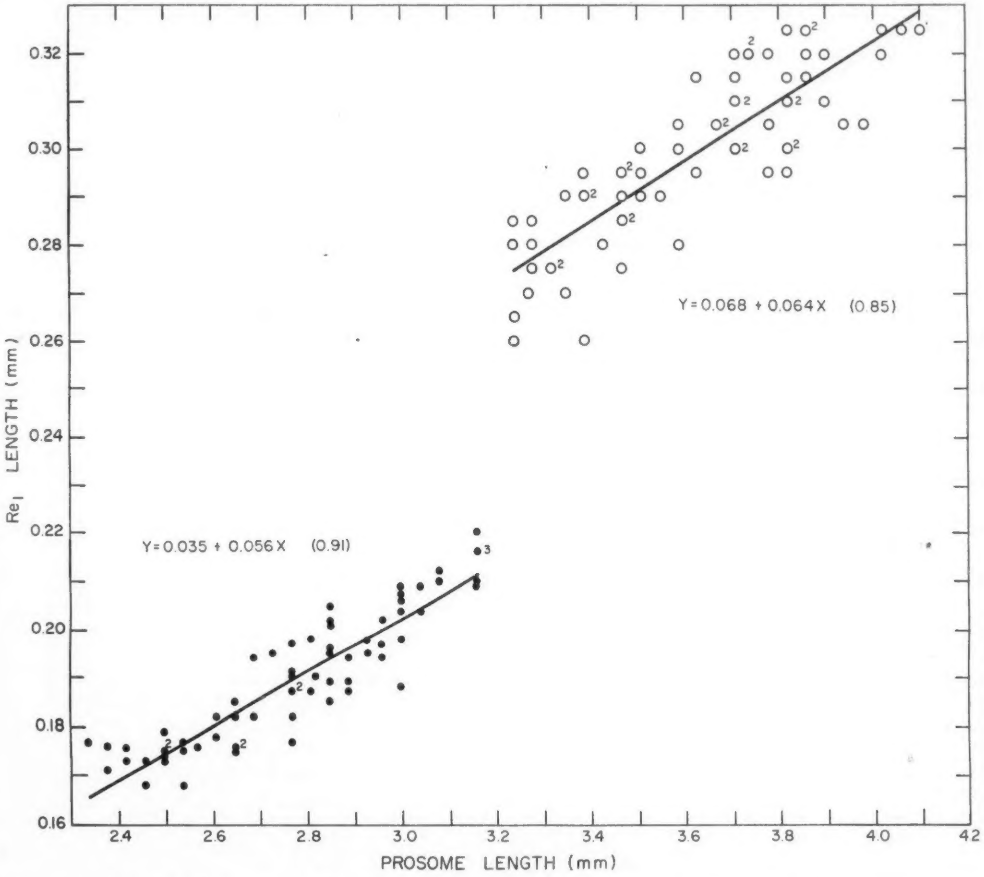

FIG. 2. Length of Re_1 of the male left fifth swimming leg plotted against prosome length (solid dots, *C. finmarchicus*; open circles, *C. glacialis*). Equations of least-squares regression lines and correlation coefficients (parentheses) are given.

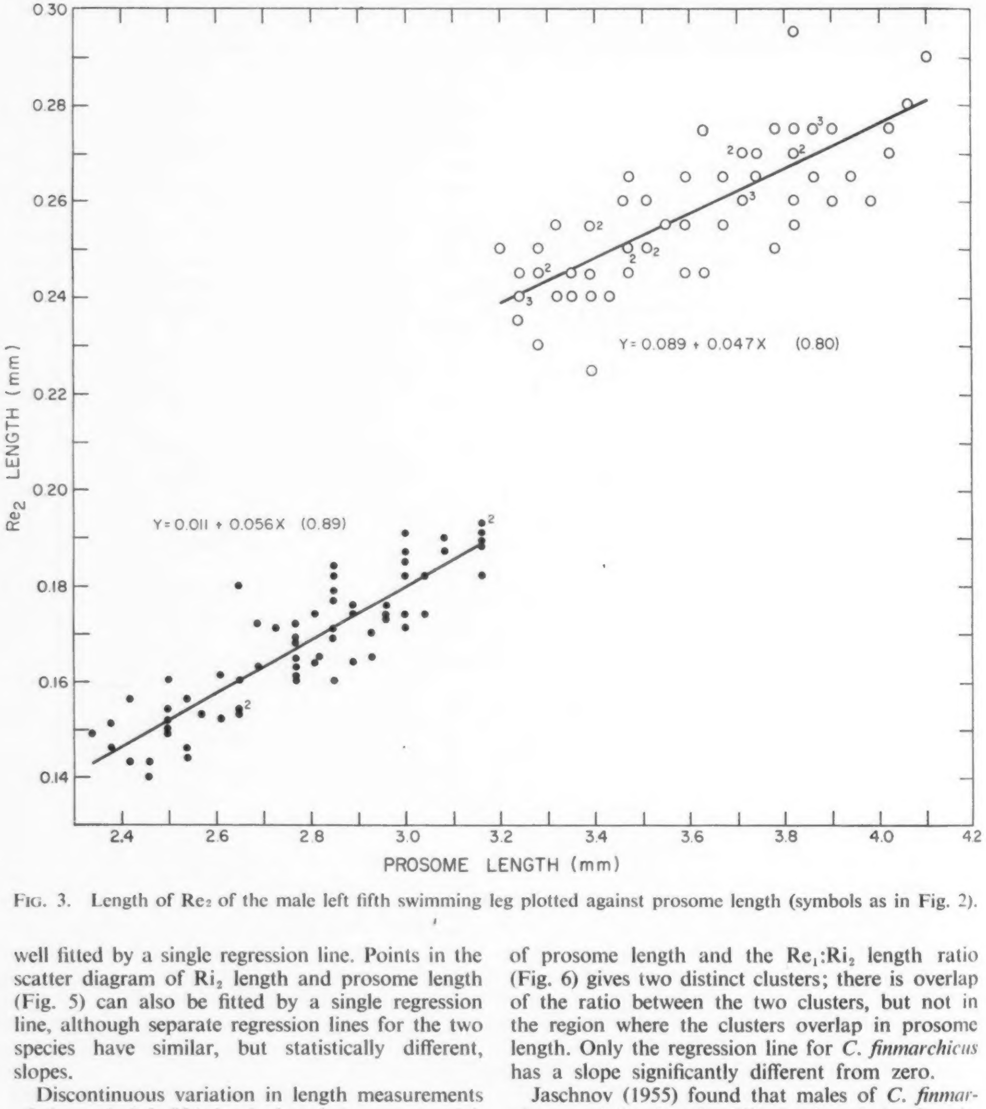

FIG. 3. Length of Re_2 of the male left fifth swimming leg plotted against prosome length (symbols as in Fig. 2).

well fitted by a single regression line. Points in the scatter diagram of Ri_2 length and prosome length (Fig. 5) can also be fitted by a single regression line, although separate regression lines for the two species have similar, but statistically different, slopes.

Discontinuous variation in length measurements of the male left fifth leg is found, in my material, only for Re_1 and Re_2. A few measurements of the right exopodal and endopodal segments showed that they vary in length in the same way as segments of the left endopod. Since the left endopodal segments do not vary discontinuously in length, comparisons of left exopodal and endopodal lengths will yield different results depending on the size of the specimens measured. For example, a plot of prosome length and the Re_1:Ri_2 length ratio (Fig. 6) gives two distinct clusters; there is overlap of the ratio between the two clusters, but not in the region where the clusters overlap in prosome length. Only the regression line for *C. finmarchicus* has a slope significantly different from zero.

Jaschnov (1955) found that males of *C. finmarchicus* and *C. glacialis* differ in the relative lengths of (1) the right and left exopods and (2) the left exopod and endopod. The fifth legs of two males with similar Re_1:Ri_2 length ratios, but differing markedly in prosome length (Fig. 1D and 1E) show that Jaschnov's criteria may be operationally difficult to apply. In the smaller specimen (Fig. 1D), the end of the left endopod extends well beyond one-half the length of the left Re_2, and the

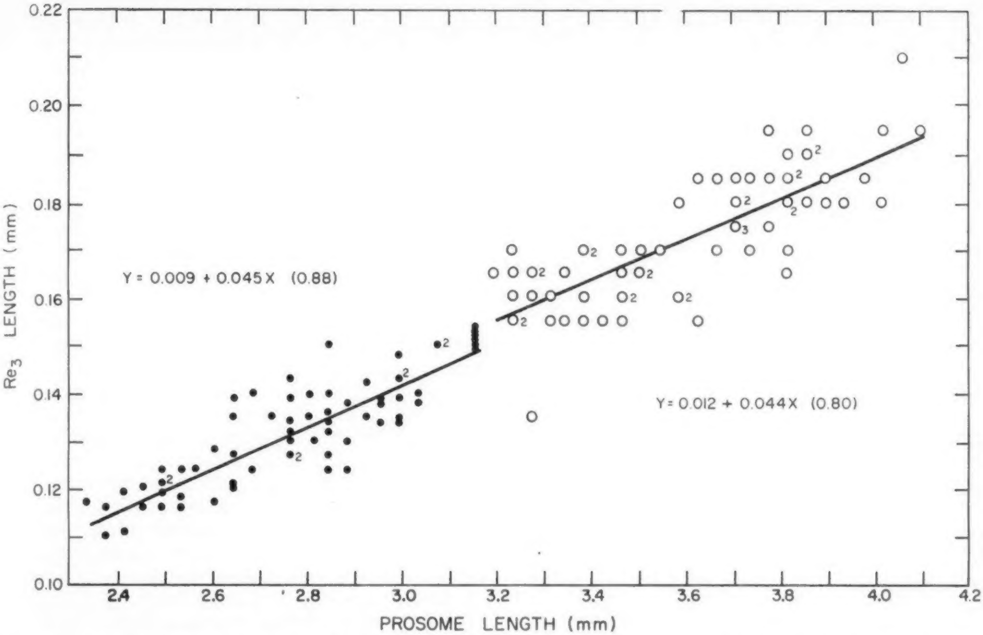

FIG. 4. Length of Re_3 of the male left fifth swimming leg plotted against prosome length (symbols as in Fig. 2). Equation of the least-squares regression line fitted to combined data for the two species is Y = 0.001 + 0.047X (correlation coefficient 0.96).

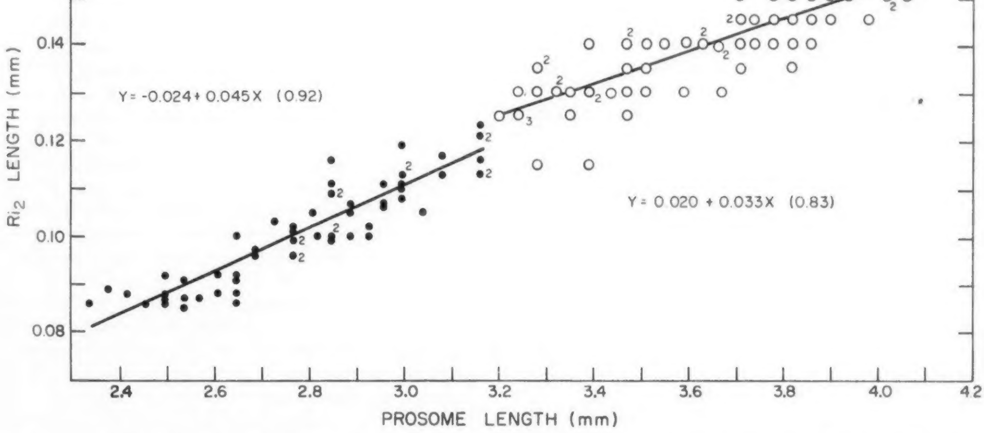

FIG. 5. Length of Ri_2 of the male left fifth swimming leg plotted against prosome length (symbols as in Fig. 2). Equation for the least-squares regression line fitted to combined data for the two species is Y = -0.020 + 0.044X (correlation coefficient 0.97).

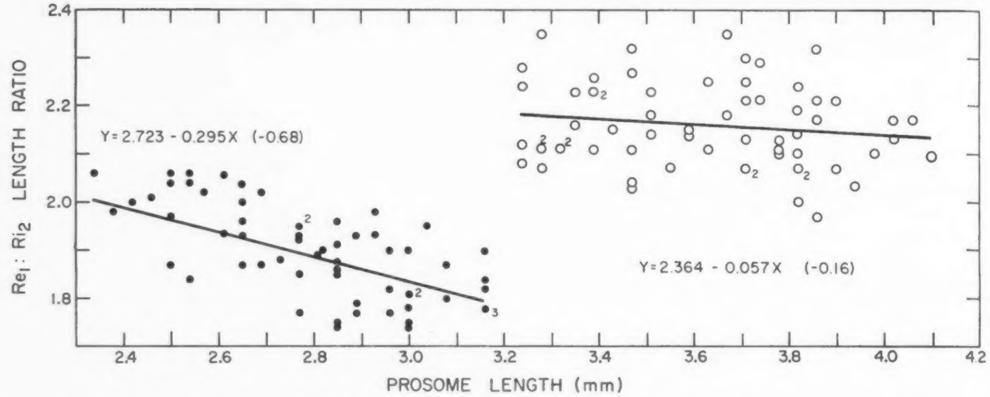

FIG. 6. Re_1:Ri_2 length ratio of the male left fifth swimming leg plotted against prosome length (symbols as in Fig. 2).

end of the right exopod reaches well beyond the articulation between the left Re_2 and Re_3. In the larger specimen (Fig. 1E), the end of the left endopod barely reaches to one-half the length of the left Re_2, and the end of the right exopod extends slightly beyond the articulation between the left Re_2 and Re_3. These are the two most similar fifth legs I have found between the two species and by Jaschnov's criteria may be referred to *C. finmarchicus* (smaller fifth leg) and *C. glacialis*. In practice, without measurements, accurate comparisons of lengths of rami are often difficult since one or both exopods may be flexed mediad or laterad. Further, Jaschnov's two comparisons are not independent, for both are based on the discontinuous variation in length of the left Re_1 and Re_2 (Fig. 2 and 3). Thus for purposes of identification it is more accurate and nearly as easy to take a measurement of the left Re_1 or Re_2, together with prosome length, and enter the coordinates in Fig. 2 or Fig. 3.

The absence of overlap in size between males of *C. finmarchicus* and *C. glacialis* is probably due to the relatively small series of specimens available. However, the separation of males between prosome lengths of 3.16 and 3.20 mm falls within the break in measurements between large and small males of "*C. finmarchicus*" found by Jespersen (1939, fig. 13). Although the largest males of *C. finmarchicus* in my material are probably very near the maximum size for that species, smaller males of *C. glacialis* might be collected from localities nearer its southern distributional limit.

Discussion

Prior to Jaschnov's work (1955), *Calanus glacialis* and *C. finmarchicus* were known as small and large forms of *C. finmarchicus*. These forms composed bimodal size-frequency distributions in series of specimens from samples collected through much of the subarctic North Atlantic Ocean. Because of the apparent lack of clear morphological differences between the large and small forms, most workers (Mrazek 1902; Størmer 1929; Jespersen 1934, 1939; Ussing 1938; Digby 1954; Wiborg 1955) thought that the forms represented a single variable species. No compelling explanation was given to account for the bimodal size-frequency distributions. Furthermore, these workers and most of those more recently concerned with *Calanus* were not experienced taxonomists, and their statements regarding a search for taxonomic characters must be viewed in this light.

For example, nearly all previous decisions on the taxonomic status of *C. finmarchicus* and *C. glacialis* are based on studies of adult females. The adult males have been little studied taxonomically, perhaps because adult males are usually much rarer in nature than adult females. Yet in many genera of calanoid copepods the males provide the most distinctive and best developed taxonomic characters. This is particularly true of male appendages and body tagmata exhibiting secondary sexual modification (Fleminger 1967; Frost and Fleminger 1968).

Males of *C. finmarchicus* and *C. glacialis* comprise two nonoverlapping clusters when Re_1 length, Re_2 length, and Re_1:Ri_2 length ratio are plotted against prosome length (Fig. 2, 3, and 6). This feature strongly suggests that populations of *C. finmarchicus* and *C. glacialis* are reproductively isolated. Further support for this interpretation is found in differences between the species in geographical distribution (Jespersen 1934, 1939; Wiborg

1955; Jaschnov 1961, 1970; Grainger 1963; Matthews 1969), vertical distribution (Jespersen 1934, 1939; Jaschnov 1958, 1961), and timing of the life cycle (Maclellan 1967; Matthews 1969). Unfortunately, I have found no other consistantly reliable character, morphological or morphometrical, to distinguish the males.

My conclusion that *C. finmarchicus* and *C. glacialis* are distinct species disagrees with the opinions of Aurich (1966) and Matthews (1967), who worked primarily with the female taxonomic characters developed by Jaschnov (1955, 1957). In this regard, the type of variation of segment lengths of the male fifth legs of the two species may provide insight into patterns of morphological variation of adult females. Jaschnov (1955) emphasized the differences between the adult females of *C. finmarchicus* and *C. glacialis* in the curvature of the medial toothed margin of the first basipodal segment of the fifth swimming leg. Both Aurich (1966) and Matthews (1967) observed intergradation between the species in this character, and Matthews showed that the degree of curvature varies with size. Variation of this character with size qualitatively resembles variation of the left Re$_3$ and left Ri$_2$ of the fifth swimming leg of adult males (Fig. 4 and 5). These female and male characters that intergrade may be best viewed as subgeneric rather than specific characters (see also Brodsky 1967a, b). Other specific taxonomic characters need to be developed for the adult females.

Aurich's (1966) proposal that *C. finmarchicus*, *C. glacialis*, and *C. helgolandicus* be regarded as geographical races of *C. finmarchicus* is not well founded. His data, based on collections from the Irminger Sea, show that for some characteristics, especially the fifth swimming leg of adult males, *C. helgolandicus* is more like *C. glacialis* than like *C. finmarchicus*. Since *C. helgolandicus* and *C. glacialis* are allopatric (Jaschnov 1961, 1970; Grainger 1963; Glover 1967; Matthews 1969), it is unclear how ecophenotypic variation could account for two races that, in some characteristics, are more similar to each other than to the *C. finmarchicus* interposed geographically between them.

I concur with Jaschnov (1955) and Brodsky (1959) that *C. finmarchicus*, *C. helgolandicus*, and *C. glacialis* are distinct species. However, the three species are imperfectly described and for now the opinions of Aurich (1966) and Matthews (1967) should be viewed with great caution by zooplankton ecologists.

Acknowledgments

I thank Dr Kr. Fr. Wiborg (Fiskeridirektoratets Havforskningsinstitutt, Bergen), Dr J. Mohr (University of Southern California), and Dr T. S. English (University of Washington) for providing plankton samples used in this study.

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Fistulicola plicatus (Cestoda) and *Tristoma* spp. (Trematoda) on Swordfish from the Northwest Atlantic

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ILES, C. 1971. *Fistulicola plicatus* (Cestoda) and *Tristoma* spp. (Trematoda) on swordfish from the northwest Atlantic. J. Fish. Res. Bd. Canada 28: 31–34.

Data on the incidence, location, and morphology of the scolex of *Fistulicola plicatus* in 24 specimens of swordfish (*Xiphias gladius*) from the northwest Atlantic are given. The occurrence of normal scolices in tubular sheaths or bladderlike cysts on the outer surface of the rectum is recorded for the first time for this species. The incidence and location on the gills of *Tristoma coccineum* and *T. integrum* are given and the possibility of using these monogenetic trematodes as biological tags is discussed. The increase in the number of dorsomarginal rows of comb teeth with body length for immature specimens of *T. coccineum* is demonstrated.

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THE swordfish (*Xiphias gladius* L.), distributed widely throughout the tropical and temperate seas, supports a valuable fishery in the northwest Atlantic. Little information is available on the morphometrics and incidence of swordfish parasites that might prove useful in solving problems of swordfish population identification and migration. In this study the identity and incidence of the parasites of the gills and gastrointestinal tract were noted and the possibility of using these parasites as biological tags was also considered.

Materials and Methods

Gills and gastrointestinal tracts of 24 swordfish caught in April, May, June, October, and December 1969 between 36–40°N and 62–74°W were preserved intact in 10% formalin (14) or deep-frozen (10). They were later dissected and examined in the laboratory for helminth parasites that were then fixed in 7% formalin. The large size of the swordfish and the fact that on commercial vessels they are dressed at sea as soon as possible after boating make the collection of parasite material difficult and samples from this source were poorly preserved.

Monogenetic trematodes were washed in distilled water, cleared and mounted in Berlese fluid prior to examination, and measured. The deep-frozen material was well extended but the formalin-preserved specimens from commercial vessels were strongly contracted and difficult to examine and measure. The cestode scolices were also cleared and mounted in Berlese fluid to aid identification.

Results and Discussion

A total of 270 monogenetic trematodes and 101 cestodes from the 24 swordfish were collected and examined, and the identity and incidence of the other helminth parasites noted (Table 1). New data on the mode of attachment of *Fistulicola plicatus* and the morphometrics of the *Tristoma* spp. are given in this paper.

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FISTULICOLA PLICATUS (RUDOLPHI 1819)

Linton (1901), Cooper (1918), and Nigrelli (1938) have previously reported this cestode from *X. gladius* from the western Atlantic. Cooper (1918) gave a detailed description of this parasite and its synonymy and noted it was frequently found piercing the wall of the intestine and rectum and that a true scolex was present only in immature forms lying free in the intestine.

In this study *F. plicatus* was found in the intestine, pyloric caecae, and rectum. The majority of the specimens pierced the wall of the rectum near the sphincter closing off the rectum from the intestine (Table 2). In some infections mature *F. plicatus* were found lying free in the rectal lumen together with other individuals that had pierced the rectal wall. Similarly, in the intestine, cestodes were found free and piercing the wall, but only one specimen was found in the pyloric caecae (Table 2).

In many instances, the scolex and much elongated neck region were found enclosed in a tough, opaque, tubular sheath (presumably produced by host tissue reaction) on the outer surface of the rectum while the strobila lay within the rectal lumen (Fig. 1A and B). Less frequently, the neck and scolex of the cestodes were enclosed in a bladderlike cyst on the outer surface of the rectum (Fig. 1A). In these instances the neck was enlarged and translucent but the two simple bothria were still visible on the scolex (Fig. 1C). In one instance, where the cestode had pierced the rectal wall, the proximal portion

TABLE 1. Location and degree of incidence of the helminth parasites. (Numbers in parentheses indicate the number of swordfish examined.)

	Location					
Parasite	Total no.	Gill cavity	Stomach	Intestine	Rectum	Pyloric caecae
Tristoma coccineum alone	167	12(24)	–	–	–	–
Tristoma integrum alone	15	3(24)	–	–	–	–
T. coccineum	66					
and		7(24)	–	–	–	–
T. integrum	22					
Fistulicola plicatus	101	–	–	5(22)	20(23)	1(22)
No. cestodes				7	93	1
Hirudinella marina	6	–	4(22)	–	–	–
Contracaecum incurvum	Numerous	–	16(22)	1(22)	–	–

TABLE 2. *Fistulicola plicatus*: incidence and location in host.

Free			Penetrated		
Pyloric caecae	Rectum	Intestine	Near sphincter	Rectal wall	Intestinal wall
1	11	2	65	17	5

of the neck was enclosed in a tubular sheath but the distal portion was dilated and translucent and lay with the scolex in a bladderlike cyst. Another specimen was found that had pierced the rectal wall, entered the body cavity, and then reentered the lumen of the rectum; the scolex was not malformed in any way. The majority of those lying free in the lumen of the alimentary tract had normal scolices but two showed malformations.

Cooper (1918) and Nigrelli (1938) had indicated that individuals of *F. plicatus* pierced the wall of the intestine and rectum but the presence of normal scolices within tubular sheaths or bladderlike cysts on the outer surface of the alimentary tract has not previously been recorded.

These modifications are probably a means of firmly anchoring the pseudophyllidean cestode with its simple unarmed scolex in a part of the gastro-intestinal tract where considerable muscular activity occurs. They suggest the production of powerful digestive secretion to enable the simple scolex to penetrate the thick muscular rectal wall; the neck may continue to extend for 3–4 cm after piercing the wall indicating a period of rapid growth, perhaps; the usual location of *F. plicatus* within the rectum may indicate a predilection for an environment free from enzymatic activity with low nutrient values being compensated for by increased absorptive powers.

Dollfus (1953) has described similar tubular structures for *Abothrium gadi* Van Beneden from the pyloric caecum of the cod, but has observed only a *scolex deformatus* in this species. Further investigations may reveal that modifications similar to those in *F. plicatus* exist in *Abothrium gadi* and other cestodes and that they are not only characteristic of *F. plicatus.*

TRISTOMA COCCINEUM CUVIER 1817 AND TRISTOMA INTEGRUM DIESING 1850

Price (1939) gave a detailed description of these two monogenetic trematodes from the swordfish of the western Atlantic and established the synonymy of *T. coccineum* Cuvier and *T. papillosum* Diesing.

Nigrelli (1938) and Linton (1940) have also recorded *Tristoma* spp. from *X. gladius* of the western Atlantic but have confused *T. papillosum*, *T. coccineum*, and *T. integrum*.

Euzet and Quignard (1961) reported *T. coccineum* and *T. integrum* from swordfish from the Mediterranean but it is interesting to note that they found much greater numbers of *T. integrum* than *T. coccineum*. This has not been recorded for

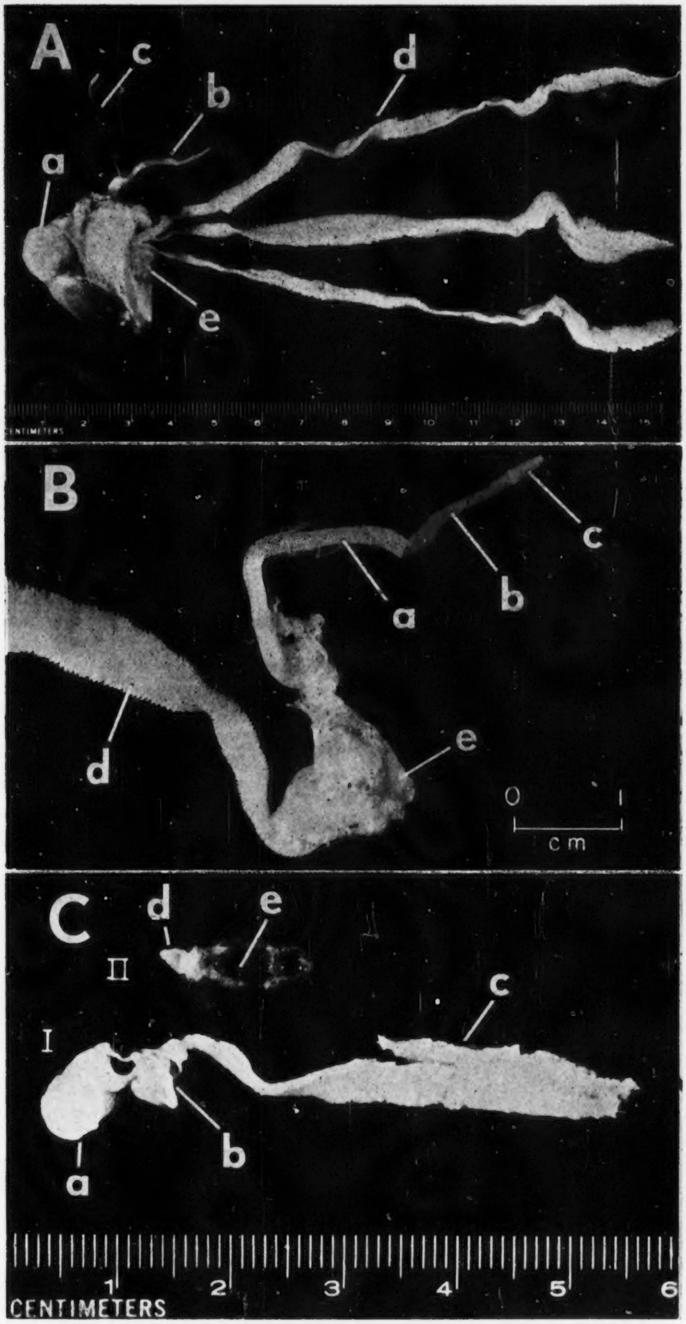
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FIG. 1. *Fistulicola plicatus* Rud. from the rectum of *Xiphias gladius*. (A) Portion of rectum with three specimens of *F. plicatus* piercing wall near sphincter muscle: (*a*) bladderlike cyst; (*b*) tubular sheath enclosing neck and scolex; (*c*) scolex and elongated neck (sheath removed); (*d*) strobila from rectal lumen; (*e*) sphincter muscle. (B) Specimens of *F. plicatus* with portion of tubular sheath removed: (*a*) tubular sheath; (*b*) elongated neck; (*c*) scolex with simple bothria; (*d*) strobila from rectal lumen; (*e*) portion of rectal wall. (C) *Fistulicola plicatus* with bladderlike cyst removed: I, entire cestode: (*a*) coiled scolex and neck removed from bladderlike cyst; (*b*) ring of rectal tissue; (*c*) strobila from rectal lumen; II, detached and flattened neck and scolex: (*d*) simple scolex; (*e*) enlarged and translucent neck.

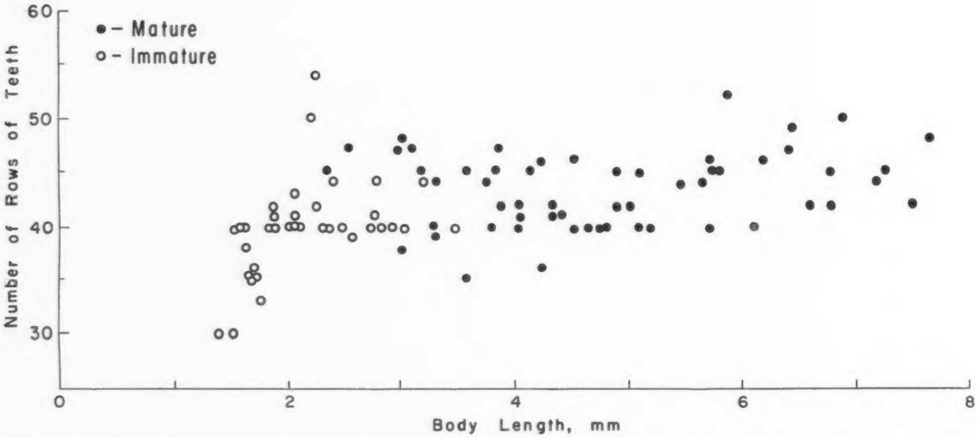

FIG. 2. Relation between body length and number of dorsomarginal rows of comb teeth (formalin-preserved specimens).

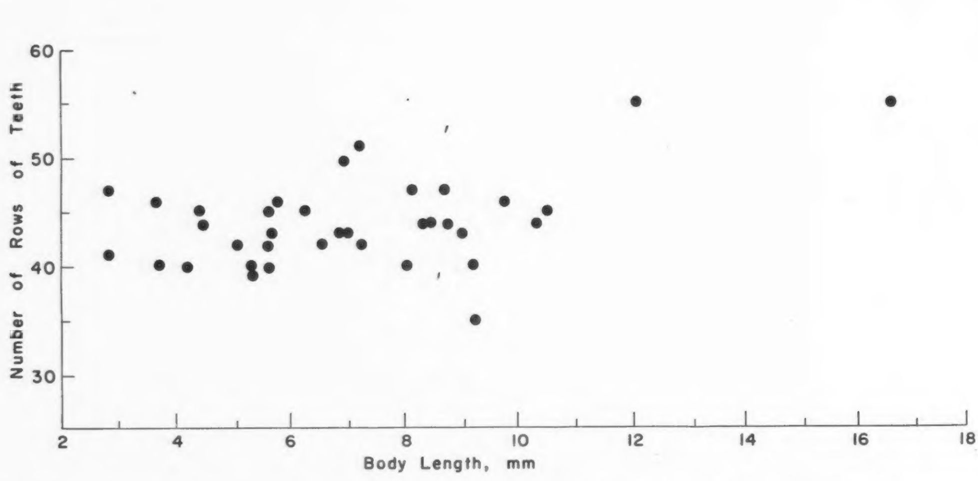

FIG. 3. Relation between body length and number of dorsomarginal rows of comb teeth (deep-frozen specimens).

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the Atlantic swordfish by Nigrelli (1938), Price (1939), Linton (1940), nor during the present study.

Yamaguti (1968) on examining *Tristoma* spp. from the gill cavity of swordfish from Hawaii erected two new species, *T. adintegrum* and *T. adcoccineum*, that he regarded provisionally as distinct from the Atlantic species as described by Price (1939).

TRISTOMA COCCINEUM RUD.

The identity of the Atlantic species *T. coccineum* was confirmed by careful examination of the 233 specimens collected during this study. They were readily distinguished from *T. adcoccineum* by the number of dorsomarginal rows of comb teeth, 30–54 for the former compared with 67–99 for the Hawaiian species. Price (1939) reported 43–54 rows for *T. coccineum* from *X. gladius* from Woods Hole but the lower end of the range found in this investigation was due to the presence of small immature specimens with fewer rows of comb teeth (Fig. 2). The strongly contracted formalin-preserved specimens from commercial vessels were smaller than those recorded by Price but the well-extended specimens preserved by deep-freezing include the size range given by Price with a comparable number of rows of comb teeth (Fig. 3). If approximately 40% contraction is allowed for the formalin-preserved specimens then the relation between body length and number of rows of comb teeth for the mature specimens is the same as that for the deep-frozen material.

The scatter diagrams (Fig. 2 and 3) for *T. coccineum* suggest that the number of rows of comb teeth increases with body length much more rapidly for immature than mature specimens. The results of the regression analysis for deep-frozen mature and formalin-preserved immature and mature specimens are given in the table below, together with the size range for each group.

	Immature			
	n	Size range (*mm*)	*r*	Slope of regression equation
Deep-frozen				
Formalin-fixed	36	1.43 ↓ 3.52	+0.43[a]	3.76 rows/mm body length

	Mature			
	n	Size range (*mm*)	*r*	Slope of regression equation
Deep-frozen	42	2.60 ↓ 16.37	+0.46[a]	0.63 rows/mm body length
Formalin-fixed	58	2.33 ↓ 7.53	+0.25[b]	0.63 rows/mm body length

[a] $0.05 > P > 0.01$.

[b] $P < 0.01$.

There is a high value for the correlation coefficient for the immature group; and the rate of increase in numbers of rows per millimeter of body length is much greater than for the mature specimens. The high value for the correlation coefficient obtained for the mature deep-frozen specimens may be generated by the outliers and may have no biológical significance (Fig. 3).

Tristoma adcoccineum is larger than *T. coccineum* (10–19 mm long × 7–15 mm wide) but the much higher number of rows of comb teeth (67–99) indicate that it is a distinct species and not a larger form of *T. coccineum*. Such high values for numbers of rows of comb teeth could not be arrived at by the extrapolation of Fig. 2 and 3.

TRISTOMA INTEGRUM

Similarly, the 37 specimens of *T. integrum* examined agreed with the description of Price in size range (3–12 mm long × 4–10 mm wide compared with 5–7 mm × 6–6.5 mm) and the presence of usually six comb teeth in each dorsomarginal row. *Tristoma adintegrum*, however, is much larger (16–22 mm long × 14–20 mm wide) with one to seven comb teeth in each dorsomarginal row (Yamaguti (1968) does not give the mean number) and is probably a distinct species.

INCIDENCE AND LOCATION

Tristoma coccineum was much more common than *T. integrum* (Table 1) although there was a much higher incidence of *T. integrum* on the small swordfish captured in December than those caught earlier in the year. It is not possible to correlate this with location, season, or age of swordfish in view of the small number of swordfish examined but further study is indicated.

The location and incidence of these monogenetic trematodes are shown in Tables 3 and 4. *Tristoma*

TABLE 3. Incidence of *T. coccineum* and *T. integrum* on particular gill arches and hemibranchs.

	T. coccineum			*T. integrum*	
	O	I	A[a]	O	I
Left					
1	2	4	5		6
2	5	10	3		7
3	2	1	18		2
4	2	0	24		1
Right					
1	6	9	3	2	6
2	4	9	11	1	4
3	7	4	25	2	5
4	1	1	15		1

[a] O: outer hemibranch; I: inner hemibranch; A: gill arch.

TABLE 4. Total numbers of *T. integrum* and *T. coccineum* on each gill, pharynx, and operculum.

	T. integrum	*T. coccineum*
1st gill		
Left	6	11
Right	8	18
2nd gill		
Left	7	18
Right	5	24
3rd gill		
Left	2	21
Right	7	36
4th gill		
Left	1	26
Right	1	17
Pharynx		
Left	0	27
Right	0	6
Operculum		
Left	0	17
Right	0	12

integrum was invariably located between two hemibranchs of a gill arch whereas *T. coccineum* was found on the gill arch, the inner surface of the operculum, pharyngeal region, on the outer surface of the hemibranchs, and very occasionally between the hemibranchs.

USE AS BIOLOGICAL TAGS

The presence of distinct species of *Tristoma* on *X. gladius* from Hawaii and the possible difference in numerical incidence of *T. coccineum* and *T. integrum* on the swordfish from the Mediterranean suggest that these monogenetic trematodes may be useful as biological tags to distinguish populations of *X. gladius*.

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This work was carried out when I was a volunteer investigator at the Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B., and I thank the members of the Pelagic Group for their assistance. I am also indebted to Dr J. S. Scott for his helpful advice on the manuscript.

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Differences in Benthos Upstream and Downstream of an Impoundment[1]

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SPENCE, J. A., AND H. B. N. HYNES. 1971. Differences in benthos upstream and downstream of an impoundment. J. Fish. Res. Bd. Canada 28: 35–43.

Pronounced differences were found in the macroinvertebrate riffle fauna upstream and downstream of a flood control impoundment. Downstream differences were comparable with those occurring after mild organic enrichment. Plecoptera were absent, but numbers of *Baetis* and *Caenis* (Ephemeroptera) increased, and the abundance and number of species of *Stenonema* were considerably reduced. Numbers of Chironomidae, Simuliidae, *Optioservus* (Coleoptera), Hydropsychidae, and *Hyalella azteca* (Amphipoda) increased downstream. These changes are associated with downstream increase in the availability of detritus, a lag of about 4 weeks in the early summer rise in water temperature and a maximum temperature more than 6 degrees C lower than upstream, and alteration of other environmental factors.

SPENCE, J. A., AND H. B. N. HYNES. 1971. Differences in benthos upstream and downstream of an impoundment. J. Fish. Res. Bd. Canada 28: 35–43.

Il existait des différences considérables dans la faune macroinvertébrée, en amont et en aval d'un barrage sur une rivière. En aval, des différences étaient comparables à ceux qu'occasionne l'occurrence d'un faible enrichissement organique: disparition complète des Plécoptères, augmentation du nombre de *Baetis* et de *Caenis* (Ephéméroptère), et diminution dans le nombre d'espèces et individus de *Stenonema*. L'accroissement en aval du nombre de Chironomidae, Simuliidae, *Optioservus* (Coléoptère), Hydropsychidae, et *Hyalella azteca* (Amphipoda) semblait lié à l'augmentation du détritus disponible et aux autres changements de l'habitat, un retard de quatre semaines environs dans le réchauffement printanier des eaux et une température maximale d'au moins 6 C. inférieure qu'en aval, et l'altération des autres facteurs d'environs.

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THE object of this study was to investigate the influence of a dam on the benthos of the river downstream. Though dams have many individual characteristics, they all impose a lentic environment on a lotic one, and in this respect the situation resembles that found in natural lake basins whose outflowing streams have biological and limnological characteristics attributable to the influence of the lake. Impoundments, however, exert influences not encountered in natural lake-river systems. The present study concerns a flood control dam in which the water is released from the hypolimnion.

Neel (1963) considered the important effects of mainstream dams to be alteration of flow patterns, reduction of turbidity, changes in temperature regime, and the development of attached masses of algae, which are so characteristic of rivers below dams.

Studies on the biological effects of impoundments on rivers have been of limited scope. Most of them deal with the downstream changes in the plankton that originated in the reservoir. Stober (1963) found that a mainstream reservoir in Montana supported an extremely low plankton population, and that the potamoplankton downstream was largely indigenous and arose from the benthic communities of the river. Coutant (1963) carried out a simultaneous study of plankton populations upstream and downstream of an impoundment when it was thermally stratified and found distinctly different populations. The plankton downstream contained many bacteria and moribund phytoplankters derived from the hypolimnion of the impoundment.

Study Area

The Shand Dam and its impoundment, Belwood Lake, on the Grand River, Wellington County, Ont., was selected for three reasons: (1) it was

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necessary to have comparable reaches of river and comparable habitats. The riffles upstream and downstream, separated by 8 km of reservoir, must have been quite similar before the dam was constructed. Temperature and flow regimes were probably nearly identical, as they have similar gradients, on a bedrock covered with glacial till, and the land bordering the river in both regions is agricultural with stands of mixed deciduous trees and white cedar. Thus, the biocoenoses were probably similar before the dam was built; (2) the dam was completed in 1942 and thus had existed for 22 years before the study started in September 1965; any long-term influence of Belwood Lake on the life cycles of fish and invertebrates in the river downstream would have become established in this time; (3) this upper region of the Grand River is virtually unpolluted and not controlled, apart from the construction of the Shand Dam.

Three physically similar sampling stations were chosen on the river (see Fig. 1). They are riffles about 7 m wide and 15–70 cm deep, depending on the flow, with beds of large stones interspersed with gravel and sand. Station 2 was chosen to exemplify the extreme effects of the dam. However, there are considerable differences superimposed on this basic physical similarity. Downstream, the substrate was always covered with dense layers of diatoms, and from June to mid-September these communities became covered by growths of *Ulothrix zonata* with associated genera of epiphytic diatoms such as *Diatoma*, *Navicula*, *Acnanthes*, *Gyrosigma*, *Cymbella*, *Gomphonema*, etc. Amongst this matrix of *U. zonata*, *Mougeotia* sp., *Nostoc sphaericum*, and *Rivularia* sp., various euglenoids, and Ciliophora were found. Above the impoundment, however, winter populations of epilithic diatoms were replaced in spring by encrusting marl-forming communities. Blum (1960) describes *U. zonata* as a rheobiont of cool, well-oxygenated streams, with population maxima in spring and late summer. The comparably cool water temperatures found downstream of the dam till mid-July (Fig. 2) partly explains its summer growth there.

The annual flow patterns at stations 1 and 2 are shown in Fig. 3. The noon water temperatures

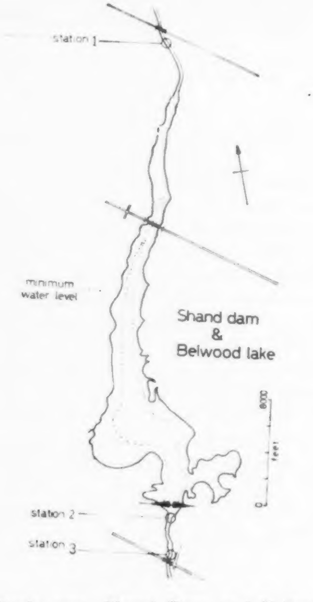

FIG. 1. Study area, Shand Dam and Belwood Lake on the Grand River, Ont., showing the three sampling stations.

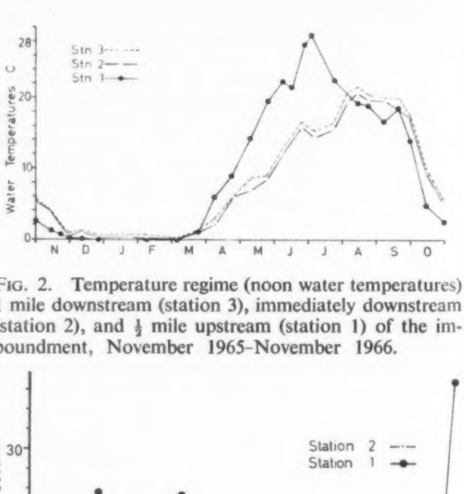

FIG. 2. Temperature regime (noon water temperatures) 1 mile downstream (station 3), immediately downstream (station 2), and ½ mile upstream (station 1) of the impoundment, November 1965–November 1966.

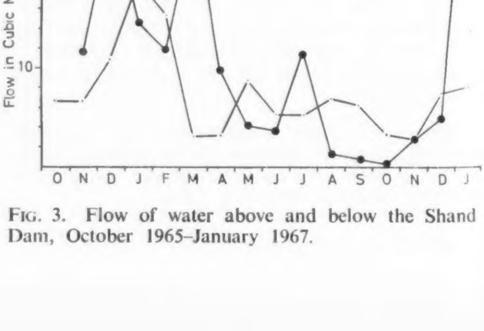

FIG. 3. Flow of water above and below the Shand Dam, October 1965–January 1967.

on sampling days at stations 1, 2, and 3 are shown graphically in Fig. 2. Upstream of the dam at station 1, the diurnal temperature cycle is characteristic of that of an exposed shallow river some distance from its source, with a minimum at dawn and maximum in the late afternoon. Thus, the present measurements made at noon are several degrees below the maximum. At station 2, the diel cycle is completely suppressed, since the outflowing water comes from the deeper strata of the lake. The only temperature fluctuation occurring there is the seasonal cycle. The same almost holds true for station 3, though there was a slight rise in the midday temperature here. There is also a considerable lag downstream in the spring rise of water temperature.

Water samples were collected at the three river stations and analysed for *p*H, bicarbonate, alkalinity, specific conductivity, organic phosphate, and nitrate nitrogen by the same methods as Duthie (1968) used in his study of the impoundment, Belwood Lake. The results are summarized in Table 1.

TABLE 1. Chemical analyses. Annual means and range of values. (Total alkalinity, phosphate, and nitrate are expressed in milligrams per liter.)

	Station		
	3	2	1
pH			
Mean	8.29	8.25	8.41
Range	7.95–8.60	7.72–8.50	7.78–9.30
Total alkalinity			
Mean	170	176	169
Range	133–205	135–205	85–227
Specific conductivity			
Mean[a]	346	350	366
Range	120–461	120–444	105–504
PO_4			
Mean	.032	.028	.035
Range	.001–.059	.001–.059	.002–.073
NO_3			
Mean	.390	.259	.225
Range	.01–.77	.01–.74	.01–.50

[a] Figures for November 1965–May 1966 only.

Considering the annual mean values of the chemical analyses (Table 1), the differences above and below the impoundment were slight. Over the year of study, there was a slight drop in *p*H, no significant difference in the levels of total alkalinity, conductivity, and phosphate, but nitrate levels were slightly higher. There was, however, less fluctuation of the various ionic concentrations below the dam, undoubtedly because of the mixing in the lake of the highly variable inflowing water.

Estimates of the total weight of suspended particulate matter and its fractions were obtained by filtration of water samples through Millipore filters of pore size 8, 0.8, and 0.22 μ. Results were expressed as milligrams air dry weight per liter.

During the summer there was more suspended matter in the river above the impoundment (Fig. 4). The decreased levels below the dam can be attributed to sedimentation and stratification in the lake. However, as the level of the lake fell during the summer and the lacustrine plankton died off, the amounts of suspended matter below the dam increased and eventually exceeded those above the impoundment, so that from mid-September to December there was several times as much below the impoundment as above. The suspended matter below the dam consisted largely of moribund plankters, in particular planktonic Cyanophyceae, that formed a succession of blooms in the lake through the summer (Duthie 1968). This increase in suspended matter downstream was probably of considerable importance to the filter feeders and other components of the communities here. The period when it occurred (September–December) is an important one for many aquatic arthropods, since it is then that most early instars have their major period of growth and possibly compete for food.

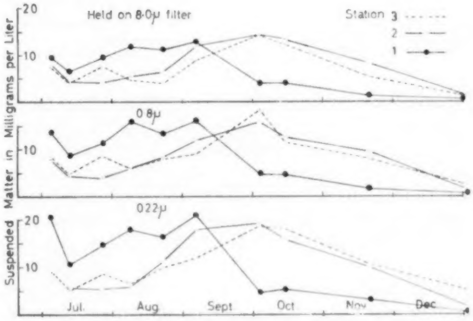

FIG. 4. Suspended matter fractions retained by membrane filters of pore size 8.0, 0.8, and 0.22 μ.

DIFFERENCES IN THE BENTHOS

The invertebrate fauna was sampled at each of the three stations by the kick sample technique of Hynes (1961) and Morgan and Egglishaw (1965). This technique is not quantitative in that it does not sample a known area of substrate, nor the animals living deep in the substrate. It is, however, sufficient to enable rough quantitative and qualitative comparisons between the three stations to be made.

For each station the numbers of the 24 major taxa occurring per sample were averaged for the year to give the average number per sample. This number, plotted in Fig. 5, it is realized, has no absolute quantitative meaning, but it does enable comparisons of the relative density of each of these 24 taxa to be made.

Differences in the ephemeropteran, trichopteran, dipteran, crustacean, and molluscan macroinvertebrates upstream and downstream of the dam are summarized in Tables 2–5. In the Odonata, nymphs belonging to four genera — *Argia*, *Ischnura*, *Calopteryx*, and *Enallagma* — were found downstream. These genera, except for *Enallagma*, were also found upstream.

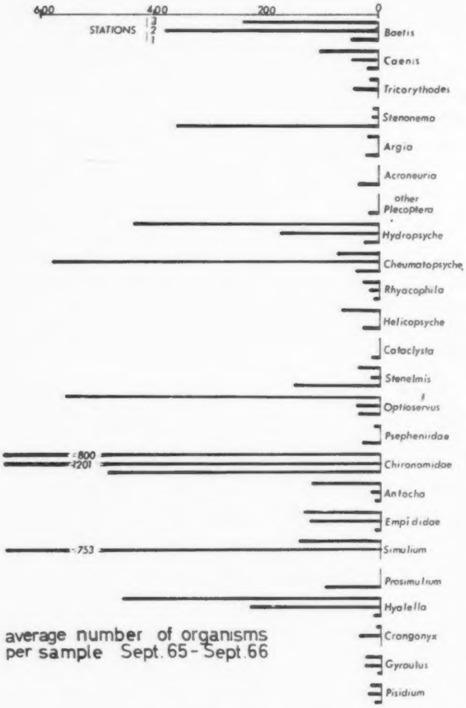

FIG. 5. Average number of organisms per sample at stations 3, 2, and 1 for the 24 main taxonomic groupings.

Among the Plecoptera, *Acroneuria lycorias*, *Paragnetina media*, and *Phasgonophora capitata* nymphs were common upstream, and during the winter *Allocapnia pygmaea*, *Taeniopteryx maura*, and *Amphinemura delosa* were also found. Plecoptera were absent, however, from the reach of river studied downstream.

In the Coleoptera, no differences were found downstream of the dam, though the Psepheniidae, represented by *Ectopria* and *Psephenus*, were absent from station 2. There were, however, noticeable quantitative differences in the distribution of the Elminthidae. Upstream, larvae of *Stenelmis* were twice as numerous as those of *Optioservus*. Downstream, *Optioservus* were four to five times more numerous than *Stenelmis*.

The only aquatic lepidopteran larvae found were those of a species of *Cataclysta* (=*Paragyractis*) at station 1 only.

TABLE 2. Differences in the ephemeropteran fauna upstream and downstream of the dam.

Species	Station 3	2	1
Baetis rusticans Mc Dunnough	+	+	–
B. levitans McDunnough	+	+	+
B. pygmaeus Hagen	+	+	–
B. vagans McDunnough	+	+	+
B. herodes Burks	–	–	+
Caenis sp.	+	+	+
Tricorythodes sp.	+	+	–
Ephemerella bicolor Clemens	+	+	+
E. deficiens Morgan	–	–	+
E. needhami McDunnough	–	–	+
Pseudocloeon sp.	–	–	+
Heterocloeon sp.	–	–	+
Paraleptophlebia mollis Eaton	–	–	+
Centroptilum sp.	–	–	+
Stenonema tripunctatum Banks	+	+	+
S. bipunctatum McDunnough	–	–	+
S. femoratum Say	–	–	+
S. canadense Walker	–	–	+
S. neopotellum McDunnough	–	–	+
S. fuscum Clemens	–	–	+
S. heterotarsale McDunnough	–	–	+
S. rubomaculatum Clemens	–	–	+
Total of 23 species	8	8	19

TABLE 3. Differences in the trichopteran fauna upstream and downstream of the dam.

Family and species	Station 3	2	1
Rhyacophilidae			
Rhyacophila fuscula Walker	+	+	+
Agapetus sp.	–	–	+
Glossosoma sp.	–	–	+
Philopotamidae			
Chimarra aterrima Hagen	+	+	+
Chimarra obscura Walker	–	–	+
Hydropsychidae			
Hydropsyche bifida Banks	+	+	–
H. slossonae Banks	+	+	+
H. betteni Ross	–	–	+
H. recurvata Banks	–	–	+
Cheumatopsyche minuscula Banks	+	+	+
Hydroptilidae			
Agraylea multipunctata Curtis	+	+	+
Myatrichia ayama Mosely	–	–	+
Ochrotrichia sp.	–	–	+
Phryganeidae			
Ptilostomus sp.	+	+	–
Limnephilidae			
Limnephilus consocius Walker	+	–	+
Neophylax autumnus Vorhies	–	–	+
Pycnopsyche lepida Hagen	–	–	+
Pycnopsyche sp.	–	–	–
Leptoceridae			
Leptocella candida Hagen	+	+	–
Athripsodes tarsipunctatus Vorhies	+	+	–
Athripsodes sp.	–	–	+
Ocoetis avara Banks	–	–	+
O. inconspicua Walker	–	–	+
Brachycentridae			
Micrasema sp.	+	+	–
Brachycentrus americanus Banks	–	+	–
Helicopsychidae			
Helicopsyche borealis Hagen	+	–	+
Total of 26 species	10	9	20

TABLE 4. Differences in the dipteran fauna upstream and downstream of the dam.

Family and species	Station 3	2	1
Tipulidae			
Antocha sp.	+	+	+
Tipula spp.	+	+	–
Pedicia	–	–	+
Dicranota	+	–	–
Empididae			
Hemerodromia sp.	+	+	+
Other species	+	+	+
Ceratopogonidae	+	+	+
Simuliidae			
Simulium vittatum Zetterstedt	+	+	–
Prosimulium fuscum Syme and Davis	–	–	+
P. mixtum Syme and Davis	–	–	+
Tanypodinae			
Procladius sp.	+	+	+
Thienemannimyia sp.	–	–	+
Diamesinae			
cf. *Pagastia* sp.	+	–	+
Orthocladiinae			
Adactylocladius sp.	–	+	–
Cricotopus sp.	+	+	–
Eukiefferiella spp.	+	+	+
Orthocladius sp. A	+	–	+
sp. B	–	–	+
sp. C	–	–	+
O. (*Euorthocladius*) sp.	+	+	+
Paracricotopus sp.	+	+	–
Trissocladius sp.	+	–	+
Chironomini			
Dicrotendipes	+	+	+
Glyptotendipes (*Phytotendipes*)	–	+	–
Microtendipes	–	–	+
Stictochironomus	+	–	+
Tanytarsini			
Tanytarsus sensu strictu spp.	+	+	+

Differences in the ostracod, water mite, and oligochaete faunas were not dealt with.

In a stream above and below a natural lake, Illies (1956) found a fourfold increase in the number of a *Baetis* sp. below the lake, and this was paralleled by increases in the numbers of *Heptagenia* nymphs, filter-feeding Trichoptera, and Simuliidae. He attributed these differences to an increase in available food. Egglishaw (1964) found a close correlation between the distribution of plant detritus and *Baetis* spp. in stream riffles, and in the present study the increase in numbers of *Baetis* spp. nymphs downstream of the dam (Table 2) can be attributed to the luxurious growths of attached filamentous algae. These provide a large number of microhabitats for the nymphs and hold more detritus than can the marl-covered stones in the river above the dam.

The large populations of *Stenonema* spp. above the lake are characteristic of clean streams in this region of North America. Ide (1935) has shown that the number of species of *Stenonema* increases

TABLE 5. Differences in the crustacean and molluscan fauna.

Family	Station 3	Station 2	Station 1
Crustacea			
Hyalella azteca Saussure	+	+	+
Crangonyx gracilis Smith	+	+	−
Cambarus bartonii Fabricus	+	−	+
Orconectes virilis Hagen	+	+	+
Total of four species.	4	3	3
Mollusca			
Sphaerium simile Say	−	−	+
S. striatinum Lamarck	−	−	+
Pisidium sp.	+	+	+
Alasmidonta calceolus Lea	−	−	+
Ferrissia rivularis Say	+	+	−
Physa sp.	+	+	+
Fossaria sp.	+	−	−
Valvata tricarinata Say	+	+	−
Helisoma anceps Minke	+	+	+
Gyraulus parvus Say	+	+	+
Total of 10 species	7	6	7

with the increasing temperature range of a stream. At station 1 the temperature range is from 0 to above 28 C and a total of eight species occurred there. *Stenonema tripunctatum* was the only species occurring downstream; it also occurred upstream. This species is known to have wide environmental tolerances, and Burks (1953) observed that it is characteristic of sluggish creeks and small silted rivers from which other *Stenonema* sp. are absent. The absence of two other *Stenonema* species from the two downstream stations would seem to be due to the lower summer temperatures found there, though the abundant Aufwuchs may also be a factor.

The absence of Plecoptera from the reach of river studied below the dam is notable. Neither the temperature regime nor the availability of food can explain the absence either of the predatory forms such as *Acroneuria*, *Phasgonophora*, and *Paragnetina* or of the herbivorous *Allocapnia* and *Taeniopteryx*. Several factors may be implicated, but most critical is likely to be the amount of oxygen available to the nymphs. The sensitivity of Plecoptera to a slight diminution of dissolved oxygen is well-documented, and Rodhe (1962) has noted that they are never found in streams that have more assimilation than dissimilation. The respiratory demand at night of heavy Aufwuchs communities can also cause oxygen exhaustion in the surface microlayers of the substrate (McIntire 1966). Such conditions are present in the downstream region of the stream studied and could explain the absence of Plecoptera.

When the Trichoptera found in the study are classified according to feeding habits, a grouping similar to that of Scott (1958) becomes apparent, that is, detritus feeders, algal scrapers, active predators, and net spinners, which are mainly predatory.

Rhyacophila fuscula, *Ocoetis avara*, and *O. inconspicua* are conventional predators (Ross 1944), and their distribution and density would be expected to be closely correlated with the availability of prey. The distribution and abundance of *R. fuscula* in the present study is clearly related to availability of prey. This is not so for *Ocoetis*, and some unknown factors related to the dam must be responsible for the absence of this genus from stations 1 and 2.

Three net-spinning families were found in the present study. The Hydropsychidae represented by *Hydropsyche* and *Cheumatopsyche* and the Philopotomidae represented by *Chimarra* occurred at all three stations. The Polycentropidae represented by *Polycentropus interruptus* occurred only upstream. The abundance of both *Hydropsyche* and *Cheumatopsyche* downstream (Fig. 3) can be related to both the availability of food and the lack of extreme current fluctuations. Although the standing crops of Hydropsychidae at stations 3 and 2 are comparable, there was a difference in species dominance at these stations. The two *Hydropsyche* species were dominant at station 3, whereas *Cheumatopsyche miniscula* was dominant at station 2. This situation undoubtedly reflects some microhabitat differences between these two stations.

The remaining Trichoptera (Table 3) are detritus and Aufwuchs feeders, but none of them, surprisingly, increased in numbers downstream of the dam. In general, the effect of the dam was to reduce the number of species and to increase the abundance of some. Out of a total of 26 species, 20 were found at station 1, 10 at station 3, and 9 at station 2. In pristine streams, the trichopteran communities show great diversity and stability. However, the ecological balance between these communities and the environment is delicate. A slight alteration of the environment could eliminate certain members of the community, and this would seem to be what has happened downstream of the dam.

Simulium vittatum was the only member of the Simuliidae below the dam, and Sommerman et al. (1954) mention that this species is characteristic of lake outflows. It can be seen from Fig. 6 that

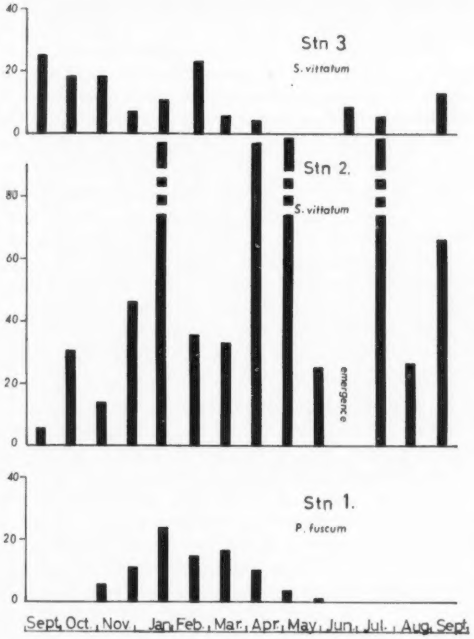

FIG. 6. Numbers of simuliid larvae per sample, September 1965–September 1966, at stations 3, 2, and 1.

it passed through two generations in the year. Overwintering larvae pupated and emerged through the latter half of April and most of May, and larvae were then absent from samples till July 15 when an abundance of minute early instars was found. These grew rapidly and were emerging in the last week of August. The numbers in the samples do not show this clearly, however, because of the staggered development rates of larvae. Davies et al. (1962) describe *S. vittatum* as being a multivoltine species and Sommerman et al. (1954) found that it had two or three generations a year in Alaskan streams, which were considerably colder (maximum of about 13 C) than the Grand River under the Shand Dam.

Prosimulium mixtum and *P. fuscum*, the only species upstream of the dam, had only one generation, which emerged in late May and early June. All species in the *Prosimulium hirtipes* complex appear to be obligately univoltine. Davies and Syme (1958) found that they had a single attenuated generation that probably spent the warm summer months in egg diapause. This would explain their total absence from samples taken between June and October but, judging from the ecological data presented by Davies and Syme (1958), there is no reason why *P. mixtum* and *P. fuscum* should not be found downstream. The temperature regime and other environmental conditions all fall within their range. The explanation would seem to be that the multivoltine *S. vittatum* builds up its numbers with summer generations so that all available suitable substrate is colonized with larvae long before the univoltine *Prosimulium* sp. have hatched.

The absence of *S. vittatum* larvae from station 1 is not due to the high midsummer temperatures reached there, since they are known to tolerate temperatures in excess of 27 C (Davies et al. 1962). It may be related to the uneven flow and scouring action of flash floods at this station.

No chironomid larvae were reared out; thus, a detailed analysis of this important group was impossible. However, even at the generic level, differences are evident.

Procladius sp., *Eukiefferiella* spp., *Orthocladius* (*Euorthocladius*) sp., and *Tanytarsus* (*s.s.*) spp. seemed to be fairly eurytypic, occurring at all three stations. One of the dominant forms upstream, *Pagastia* sp., did not occur in the extreme conditions of station 2, but reappeared at station 3. Three species of *Orthocladius* (*s.s.*) were confined to station 1 upstream, and *Cricotopus* sp. and *Eucricotopus* sp. were associated with the growths of Aufwuchs downstream.

CRUSTACEA

Hyalella azteca, though abundant downstream, occurred only sporadically upstream of the dam. *Hyalella* is a eurythermal detritus feeder, and its abundance downstream can be explained by the availability of microhabitats and food amongst the algal growths.

Crangonyx gracilis occurred only downstream of the dam. This restriction would seem to be due to the cooler temperatures found here in the summer. Judging from ecological notes in Bousfield (1958), *C. gracilis* is a cold stenothermal species. Downstream, the temperatures exceeded 20 C only for a short time in August, whereas upstream of the impoundment the noon temperature exceeded 20 C during June, July, and early August, reaching a recorded maximum of 29 C in early July. *Crangonyx gracilis* was most abundant at station 2. This may reflect an accumulation of upstream migrating individuals whose progress was impeded by the dam. Such upstream movements have been reported before by Hynes (1955) who noted that small dams prevented the upstream movements of *C. pseudogracilis* Bousfield, and by Minckley (1964) for several *Crangonyx* species.

MOLLUSCA

Most of the gastropod species recorded are not found in the rapid areas of streams but are usually associated with shoreline vegetation and backwaters. Thus, little can be inferred from their occurrence in the present samples. One exception is the ancylid *Ferrissia rivularis*, which was found only below the dam. It seems to be a species that is assisted by human interference with its environment. Goodrich (1932) noted that it occurred in great numbers below the Superior dam on the Huron River, Michigan, whereas other molluscs that had once been common were absent.

The Pelecypoda, however, were considerably affected by the dam. Only one, a *Pisidium* species, occurred below it although *Sphaerium striatinum*, common above, and the naiad *Alasmidonta calceolus* did not. Stream-dwelling naiads are known to be sensitive to human interference (cf. van der Schalie 1938, and others), but the reasons are not clear. Pollution, substrate changes, alteration of flow patterns, and loss of fish hosts of the glochidia are undoubtedly implicated. One of the two known fish hosts of *A. calceolus* is *Cottus bairdi* (Clarke and Berg 1959), which occurs above but is more abundant below the impoundment. Thus, the absence of *A. calceolus* cannot be explained by lack of a specific glochidial host; possibly there is some effect related to the dense growths of Aufwuchs.

Discussion

Having assumed that stations 1, 2, and 3 sustained similar biocoenoses before 1940, it is evident that construction of the dam has caused great changes in the community structure of the benthos. This is reflected particularly in the Ephemeroptera and Trichoptera, in which three types of changes are discernible downstream: a reduction in the total number of species (see Tables 2–5), an increase in the numbers of some species (see Fig. 3), and the replacement of yet other species by closely related ones (see Tables 2–5). These faunal changes are the result of alterations of the stream environment by the dam. Physical, chemical, and biological factors could be involved. It is highly unlikely that the slight variations in water chemistry exerted any influence on the distribution of the fauna. At all times the *p*H was above 7 and the range of variation of cationic concentrations was much less than that that is considered critical in the literature. Macan (1955) found only vague correlations between the ionic concentrations of lakes and their invertebrate faunas. His conclusion, that the distribution of the fauna would show a correlation to the more particular conditions of a habitat, can be applied here.

The difference in temperature regime is considered to be of importance. It can explain the distribution of the *Stenonema* species as discussed and of some of the fish species (Spence and Hynes 1971), but it is impossible to ascertain its effect on the other invertebrate species in the absence of data on their temperature requirements and other environmental factors. The remaining investigated effects are all biological in nature, such as the downstream development of attached algae and the increase in suspended matter during certain seasons. The increases downstream in the density of *H. azteca*, *Caenis* sp., *Baetis* spp., *Optioservus*, and Chironomidae are undoubtedly related to the greater availability of detritus and the more uniform flow here.

In attempting to answer the question of what factors caused the biocoenotic changes below the dam, one must remember that many environmental factors affect invertebrates and that several are interrelated. It is, therefore, perhaps rash to single out a few as responsible for the changes, but at least three primary factors are important: (1) alteration of the temperature regime so that water temperatures are considerably lower in summer; (2) abundant development of Aufwuchs, partly resulting from lower summer temperatures and even discharge, and involving radical alteration of the substrate surface; and (3) outflow of organic matter, particularly zooplankton and phytoplankton, from the impoundment.

The significant point of this study is, however, that collectively these factors tend to change the benthos in the same general way as does mild organic pollution.

Acknowledgments

We thank Dr O. A. Sæther of the Fisheries Research Board of Canada, Freshwater Institute, Winnipeg, Man., who identified some of the Chironomidae larvae, and the Grand River Conservation Authority for their cooperation.

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Differences in Fish Populations Upstream and Downstream of a Mainstream Impoundment

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Four species of cyprinid fishes, *Hybopsis biguttata*, *H. micropogon*, *Pimephales notatus*, and *Notropis spilopterus* found in a river upstream of a flood control dam are absent downstream. With the release downstream of cold hypolimnial waters from the dam there was a lag of about 4 weeks in the spring rise of water temperatures and a maximum summer temperature 7 degrees C lower than upstream. Of the fish present in the river these four species had the most southerly distributions, all south of the 64 F (17.8 C) July isotherm, and it is concluded that the lower water temperatures are mainly responsible for their absence downstream of the dam.

SPENCE, J. A., AND H. B. N. HYNES. 1971. Differences in fish populations upstream and downstream of a mainstream impoundment. J. Fish. Res. Bd. Canada 28: 45–46.

Quatre espèces de Cyprins, *Hybopsis biguttata*, *H. micropogon*, *Pimephales notatus* et *Notropis spilopterus*, bien qu'abondantes en amont d'un barrage servant au contrôle des crues, faisaient complètement défaut en aval. On a constaté, par rapport à l'amont, un délai de quatre semaines dans la réchauffement printanier des eaux; de plus, la température maximale enrégistrée en été était de 7 C. supérieure en amont. De toutes les espèces de poissons dans la rivière, on s'apercoit que ces quatre espèces absentes en aval sont celles qui ont les répartitions les plus méridionales, toutes situées au sud de l'isotherme de juillet de 64 F. (17.8 C.); les températures plus basses en aval du barrage semblent responsables de cette absence.

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IN a study of the differences in stream benthos upstream and downstream of an impoundment (Spence and Hynes 1971) we found that changes in the benthic communities were attributable to several ecological effects of the dam. Samples of the fish present in the river upstream and downstream of the dam were taken by electrofishing, seining, and hand net between September 1965 and January 1967 (Table 1). There are considerable differences in the fish species occurring in these parts of the river now separated by the 8-mile-long impoundment. Prior to the construction of the dam in 1940, environmental conditions in these two parts of the river were similar, if not identical, and their fish populations were contiguous. Thus, the present differences must largely be consequent to the construction of the dam.

In 1966 the noon water temperature (several degrees below the daily maximum) above the impoundment reached 16 C in the 2nd week of May and a maximum of about 28 C in June, whereas below the dam 16 C was not exceeded until the 2nd week of July and the maximum was 21.5 C in the middle of August (Spence and Hynes 1971, fig. 2). These temperature differences can explain the absence of several cyprinid species below the dam. Miller (1964) found that cyprinid minnows in three New York streams did not start nest-building behaviour in spring until the daily maximum water temperature was above 16 C, and if the water temperature then fell below this all breeding activity ceased temporarily. He found that breeding activity was continuous from the end of April to July through the water temperature range of 16–27 C and that spawning behaviour in these species was triggered at different temperatures: *Semotilus atromaculatus* began at the end of April, when the maximum water temperature was 16 C; *Notropis cornutus* was next; then *Hybopsis micropogon* several weeks later when temperatures ranged from 16 to 26 C.

These three species were also found in the Grand River, but only *H. micropogon* (with the highest temperature threshold) was absent downstream of the dam. In general, the breeding of all cyprinid species would be considerably delayed under the dam. In *S. atromaculatus* and *N. cornutus* this delay was not critical, but it would appear to have disrupted the life cycles of *Hybopsis biguttata*, *H. micropogon*, *Pimephales notatus*, and *Notropis spilopterus* downstream of the dam.

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TABLE 1. Fish species taken downstream of the Shand Dam and above its impoundment, Belwood Lake.

	Down-stream	Up-stream
Catostomidae		
Catostomus commersonii Lacépède	+	+
Cyprinidae		
Semotilus atromaculatus Mitchill	Common	+
Chrosomus eos Cope	+	+
Pimephales notatus Rafinesque	–	+
Notropis cornutus Mitchill	+	Common
N. spilopterus Cope	–	+
Hybopsis biguttata Kirtland	–	Common
H. micropogon Cope	–	+
Rhinichthys atratulus Herman	+	+
R. cataractae Valenciennes	–	+
Centrarchidae		
Ambloplites rupestris Rafinesque	+	+
Percidae		
Perca flavescens Mitchill	Common	+
Cottidae		
Cottus bairdi Girard	Common	+

Further evidence that the absence of these cyprinid species downstream of the dam is due to alteration of the temperature regime was obtained by comparing the northern limits of the known distributions of *H. biguttata*, *H. micropogon*, *N. spilopterus*, *P. notatus*, and *Notropis cornutus frontalis* in eastern Canada in relation to the July isotherms (Fig. 1). The northern limits of distribution are based on data from Radforth (1944), Scott (1954), Keleher (1956), Hubbs and Lagler (1958), and Fowler (1961). The remaining cyprinids, *S. atromaculatus*, *Chrosomus eos*, *Rhinichthys atratulus*, and *Rhinichthys cataractae*, and the other fish species all have distributions that extend north of that of *N. c. frontalis*. The four cyprinid species with the most southerly distribution, *H. biguttata*, *H. micropogon*, *P. notatus*, and *N. spilopterus* are absent below the dam. All have a distribution south of approximately the 64 F (17.8 C) isotherm. The one exception is *R. cataractae*, found above but not downstream of the dam. It was a rare species above the dam and its absence downstream may be due to some undetermined ecological effect of the dam, or possibly to interspecific competition with *Perca flavescens*, *S. atromaculatus*, or *Cottus bairdi*, all of which increased greatly in numbers downstream of the dam.

The important effect of the dam has been to eliminate a number of southern species of fishes. This is likely to be a general effect of the ever-increasing number of small flood-control and summer water-level maintenance dams that are being constructed or proposed in eastern North America.

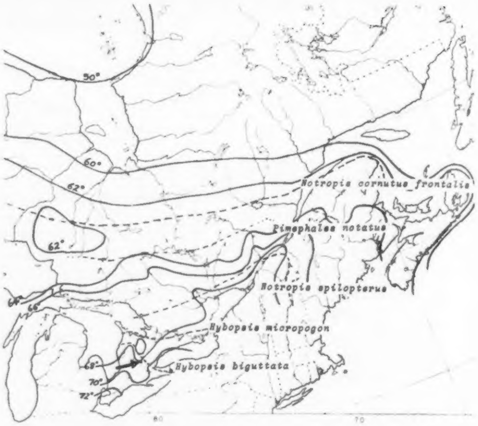

FIG. 1. July isotherms across eastern Canada (solid lines) and approximate northern limits of distribution in eastern Canada of five species of cyprinid fishes (broken lines). The data for the July isotherms were taken from Brown et al. (1968) and Chapman and Thomas (1968).

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Relation of Hemosiderin to Erythropoiesis in the Blue Gourami, *Trichogaster trichopterus*

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YU, M.-L., C. W. KILEY, D. A. SAROT, AND A. PERLMUTTER. 1971. Relation of hemosiderin to erythropoiesis in the blue gourami, *Trichogaster trichopterus*. J. Fish. Res. Bd. Canada 28: 47–48.

The presence of hemosiderin bodies was demonstrated in the spleen, liver, and kidney of the blue gourami, *Trichogaster trichopterus*, with the spleen as the predominant site of accumulation. Bleeding, which indirectly stimulates erythropoiesis in this fish, reduced the percentage of hemosiderin bodies in experimental fish to a much lower value than was found in normal fish. A possible relation between these hemosiderin bodies and erythropoiesis is discussed.

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RECENT work (Zanjani et al. 1969) indicated that the rate of erythropoiesis in the blue gourami, *Trichogaster trichopterus*, was controlled by a circulating factor that is similar in function to the erythropoiesis stimulating factor found in mammals (Gordon 1959), birds (Rosse and Waldmann 1966), and amphibians (Rosse et al. 1963). In the blue gourami, injection of anemic fish serum, or bleeding or hypoxia, resulted in increased erythropoiesis as indicated by higher hematocrit and hemoglobin values. An increased rate of radio-iron incorporation also accompanied these responses. The increase of erythropoiesis was achieved within 48–72 hr (Zanjani et al. 1969). It is evident that during this short period enough iron must be available to the fish to synthesize the hemoglobin for new red blood cells. The present investigation was undertaken to determine whether the kidney, spleen, and liver of this fish contain hemosiderin, a storage form of iron found in the spleen of the bluegill sunfish (Grover 1968), and to determine the possible role played by hemosiderin in erythropoiesis.

Materials and Methods

TRACER STUDIES

Adult male and female fish weighing 6–8 g were used in all experiments. Radio-iron labelled ($Fe^{59}Cl_3$) red blood cells were obtained from 15 fish, using the technique of Zanjani et al. (1969). The blood from these fish (0.75 cc) was pooled, washed four times in saline, and resuspended in an equal volume of saline. A 0.05-cc sample of this suspension, registering approximately 7000 counts/min was injected into each of six untreated normal fish by means of the technique of Yu et al. (1970). The fish were killed 9 days later and spleens, livers, and kidneys were removed. These tissues were separately pooled (i.e., all livers together) and the radioactivity in each group was determined by using a well-type scintillation counter.

HISTOLOGICAL STUDIES

To stimulate erythropoiesis in the blue gourami, 0.05–0.08 cc of blood was removed from each of 30 fish by means of a 0.25-cc syringe and a 27-gauge needle. The needle was inserted in the midline of the jugular region of the fish at a point approximately 5 mm posterior to the apex of the V formed by the ventral cover of the opercula (Zanjani et al. 1969). No anesthetics were used in the bleeding operation.

The bled fish were divided into three groups of 10, and six untreated normal fish acted as controls. All animals were fed equal amounts of a mixture of cooked beef liver and baby oatmeal (Beechnut Co., New York, N.Y.). They were kept in 2.5-gal disposable plastic aquaria, and maintained at room temperature (24–28 C). The first group of experimental fish was killed by decapitation immediately after bleeding. The second and third groups were decapitated 24 and 48 hr after bleeding, respectively. Spleens, livers, and kidneys were immediately removed and fixed in Helly's solution for 4 hr, washed in running distilled water for 22 hr, dehydrated in ethyl alcohol, embedded, and sectioned at $9\ \mu$. The six control fish were killed with the first experimental group, and their spleens, livers, and kidneys were treated in the same manner.

Half of the tissue sections from each group was stained (Dominici 1902), and the other half was tested for the presence of hemosiderin by Perl's reaction. Wood and Yasutake (1956) reported the presence of a ceroid in fish spleen that had the same pigment pattern as hemosiderin, but that fluoresced under ultraviolet light. To differentiate between possible ceroid and hemosiderin areas, stained sections of tissue were exposed to ultraviolet light and only the hemosiderin areas recorded. Stained tissues were projected and camera lucida drawings

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made of section and hemosiderin areas. The percent of the area occupied by hemosiderin in the different tissues was determined with a planimeter, and the percent and size of hemosiderin in controls and experimental animals was compared.

Results

TRACER STUDIES

The percent distribution of radio-iron ($Fe^{59}Cl_3$) in the livers, spleens, and kidneys of six fish is shown. The spleens were the predominant site of radioactivity accumulation.

	% of injected label			
	Spleen	Kidney	Liver	Other tissues
Normal fish	55	18	6	21

HISTOLOGICAL STUDIES

Examination of stained sections of spleens, livers, and kidneys from six control fish revealed large green clusters, apparently composed of smaller bodies. The mean percentage of hemosiderin and standard errors of the means were: spleen, 8.60 ± 1.200; kidney, 0.02 ± 0.005; liver, 0.05 ± 0.010.

These clusters were dispersed throughout the spleen; some were adjacent to, or in the lumen of the splenic vessels (Fig. 1, upper). Perl's test indicated that these clusters were indeed hemosiderin. Furthermore, they did not fluoresce under ultraviolet light as did ceroid pigments.

Sections of spleen taken from six experimental fish 24 and 48 hr after bleeding had a lower percentage of hemosiderin. Spleen had 4.38 ± 0.9 after 24 hr, 3.61 ± 0.5 after 48 hr. Kidney and liver sections showed no hemosiderin clusters after 24 and 48 hr.

The percentage of hemosiderin in liver, spleen, and kidney of the experimental group killed immediately after bleeding was the same as that in control fish. However, many of the large hemosiderin bodies appeared to be breaking down into smaller bodies (Fig. 1, lower).

Discussion

Bessis and Breton-Gorius (1959) demonstrated that when dying human erythrocytes were phagocytized in the spleen, ferritin was produced and later incorporated by different cells by a process of rhopheocytosis (ferritin uptake). Orlic et al. (1965), using electron microscopy, demonstrated that as early as 12–24 hr after erythropoietin injection into polycythemic rats, the basophilic erythroblasts in the spleens begin to take up ferritin. The injection of radio-iron-labelled red blood cells into the blue gourami resulted in a greater concentration of iron in spleen than in liver or kidney. This may be due to either the accumulation of radio-iron that has escaped from circulating red blood cells, or the destruction of labelled red blood cells in the spleen. Work in progress using radioactive chromium (Cr^{51}, Mollison and Veall 1955) indicates the latter possibility may be true. These results indicate that the spleen is possibly the site where dying red blood cells are phagocytized and their iron stored as hemosiderin. However, within 24–48 hr after bleeding, which indirectly stimulates red blood cell formation (Zanjani et al. 1969), the hemosiderin bodies were broken up into small bodies that were then taken up by splenic cells (Fig. 1). It is conceivable that these hemosiderin bodies are either used by the spleen, or transported to other hematopoietic organs for the synthesis of new red blood cells.

Acknowledgments

This work was supported in part by grant UI-00327-03 from the U.S. Public Health Service.

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(FIG. 1 *opposite*)

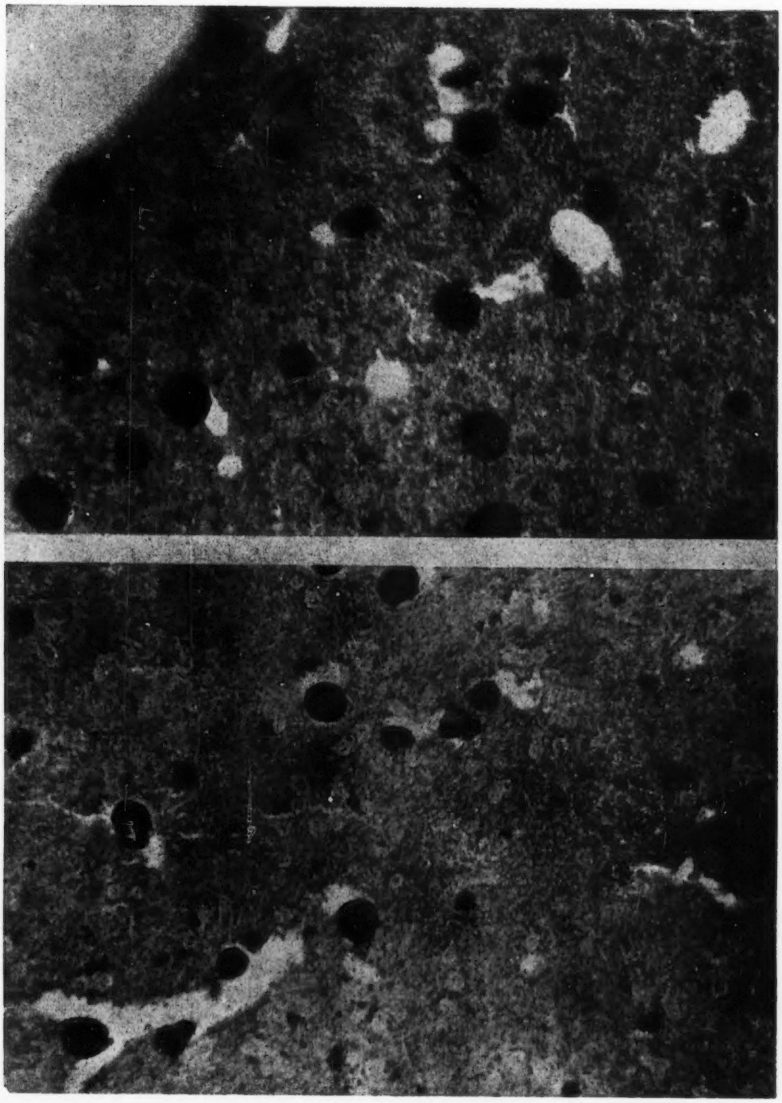

FIG. 1. Section of spleen with hemosiderin bodies (Dominici strain). ×100. *Upper*, control; *lower*, 48 hr after the bleeding operation.

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Comparison of Size, Structure, and Distributional Patterns of Two Salamander Populations in Marion Lake, British Columbia[1]

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NEISH, I. C. 1971. Comparison of size, structure, and distributional patterns of two salamander populations in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 28: 49–58.

During 1968 and 1969, populations of *Taricha granulosa* and neotenous *Ambystoma gracile* were trapped and observed in Marion Lake, B.C. The adult *T. granulosa* and *A. gracile* populations were estimated respectively at 2450 and 14,500. It was estimated that there were about 45,000 *A. gracile* young of the 2nd year in the lake. These populations represent total lake biomasses of about 18 kg for *Taricha* and 408 kg for *Ambystoma*. The numbers of young larvae were not determined for either species. The *A. gracile* population was widely dispersed throughout the spring and summer. Density and size composition varied between areas with different vegetation and substratum. The *T. granulosa* distribution within the lake was highly contagious and changed through time. Although displaced animals returned to the areas where they were caught, the positions of their home ranges changed through time. *Taricha granulosa* entered the lake in April and May, showed evidence of wandering in midsummer, and left the lake in September and October. Fifty percent of adult *A. gracile* seen during the breeding season were metamorphosed and were found in the lake only during April and May. Neotenous adults remained in the lake all year, as did larvae of the 1st and 2nd years. Those larvae that metamorphosed did so in late August and early September. *Ambystoma gracile* grew rapidly in the lake, but no growth was seen in *T. granulosa*. No source of *T. granulosa* mortality was found in the lake, but *A. gracile* were preyed on by fish and diving bugs, and dead specimens were found on the lake bottom.

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MARION Lake, British Columbia, contains four predacious aquatic vertebrates. Two of these are salmonid fish, the rainbow trout (*Salmo gairdneri* (Richardson)) and the kokanee (*Oncorhynchus nerka* (Walbaum)). The other two are salamanders. One of these is the newt (*Taricha granulosa* (Skilton)) and the other is a neotenous form of *Ambystoma gracile gracile* (Baird). The larvae of both species also occur in the lake. As part of a continuing study on energy flow in Marion Lake, an intensive study is being made of predation by fish and salamanders.

An accurate assessment of the population structure and movements of both salamanders was necessary before their impact as predators could be established. Efford and Mathias (1969) have published the results of a study on this subject. The present paper describes the results of a continuation of their study.

Methods

THE STUDY AREA

Marion Lake is a small mud-bottomed lake situated 10 km north of Haney, B.C. It is about 10 ha in area during the dry summer season, when most of the present work was done. The lake is situated at about 300-m elevation in a north–south valley heavily wooded with secondary coniferous forest. For a detailed description of the lake see Efford (1967).

A substratum map of the lake was made during a diving survey (Fig. 1). Seven substratum types were defined in terms of water depth and vegetation type.

"Open mud" areas of four water depths had a substratum of sediment containing sticks and leaves. During the summer a sparse cover of filamentous algae occurred in open mud areas deeper than 1 m. Shallower areas were covered with a thick mat of algae.

The substratum type referred to as "submerged vegetation" included areas having a growth of *Potamogeton epihydrus* (Raf.), *Chara globularis* (Thuill), and *Isotes occidentalis* (Henders.). These plants formed a mat on the lake bottom.

Potamogeton natans (L.) and *Equisetum* sp. are species of emergent plants that often occurred together, so areas containing these species were considered as one substratum type.

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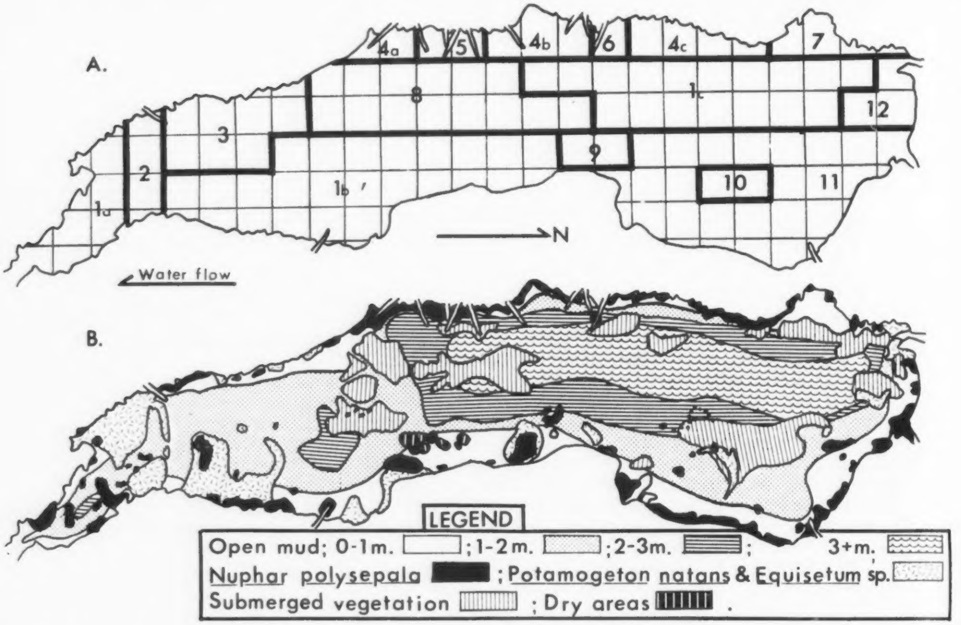
A.
4a
5
4b
6
4c
7
8
1c
12
3
9
2
10
11
1b
1a
N
Water flow
B.
LEGEND
Open mud; 0-1m. ; 1-2m. ; 2-3m. ; 3+m.
Nuphar polysepala ; Potamogeton natans & Equisetum sp.
Submerged vegetation ; Dry areas .

FIG. 1. Marion Lake: (A) the grid system shown was marked in the lake by means of numbered floats at the intersection of transects. The areas enclosed in heavy lines are referred to in Fig. 4 in the text; (B) substratum types, defined in terms of vegetation and water depth.

The largest emergent macrophyte in Marion Lake was the lily pad *Nuphar polysepala* (Engelm.). These plants grew in widely spaced clumps with open mud between them. In some instances *P. natans* grew among *N. polysepala*.

In Marion Lake the sizes and shapes of *P. natans*, *I. occidentalis*, *N. polysepala*, and *Equisetum* sp. beds changed from year to year. This can be clearly seen if Fig. 1 in this paper (showing 1969 data) is compared with Davies' 1966 data (Davies 1970).

TRAPPING AND OBSERVATION

During the months of July–October 1968, and April–October 1969, *T. granulosa* and *A. gracile* were observed by the following methods:

(*a*) A set of floodlights was mounted on the stern of a rowboat. At night when the lake surface was calm both species of salamander could be seen in water not deeper than 3 m. From the boat *Taricha* could be captured with a dipnet. *Ambystoma* were able to escape the dipnet except when the water was colder than 10 C. This species was usually caught using a "slurp gun," which is a syringe-like device about 1 m in length with a spring-loaded plunger. During the 1969 study period the portion of the lake less than 3 m in depth was surveyed from a boat once per week.

(*b*) During July, August, and September 1968, 75 wire mesh funnel traps were distributed around the lake and were checked every 2 days. In 1969, funnel traps were placed in the lake according to a grid pattern (Fig. 1). The grid consisted of 114 traps placed at the corners of 1000-m² quadrats. Auxiliary traps were placed along the shoreline, giving a total of 132 traps. These were all checked five times per week during the period May 1–September 15 and twice per week until mid-October. The trapping program was discontinued at this time because few *Ambystoma* and no *Taricha* were being caught.

(*c*) Once per week from May 1 to September 15, 1969, the entire lake was surveyed with the use of skin-diving and scuba diving gear. *Ambystoma* could not be easily captured by a diver, but many *Taricha* were caught by hand.

MARKING

All *Taricha* captured by the above methods were marked with individual toe clips. Immediately after capture the snout–vent measurement of each animal was recorded, its sex was determined, and if the animal was caught for the first time it was marked. The animal was then released at the site of capture, usually within a few minutes of being removed from the water.

Individual marking of *A. gracile* was done only during the summer of 1968. The regenerative powers of larval and neotenous *Ambystoma* were such that toe clips could not be used. It was therefore decided to loop color-coded threads around the gill rakers. This was done to 504 animals in the lake. Laboratory animals marked in this way did not lose their marks, but the method was so time consuming and the returns were so low (16 recaptures) that this program was not continued in 1969. All *A. gracile* captured in grid traps were measured and the *Ambystoma* catch record of each trap was kept. The sex of neotenous *A. gracile* could not be accurately determined.

POPULATION ESTIMATES

The size of the *Taricha* population was determined using the mark-recapture results of 1969. The estimate was calculated using the Schumacher modification of the Schnabel estimator (Ricker 1958, equation 18, p. 101). In view of the difficulty encountered in marking *Ambystoma*, it was decided to estimate the size of the *A. gracile* population using the following method:

(*a*) All *A. gracile* caught in the Marion Lake trap grid were counted and (after June 16) their snout–vent lengths were measured.

(*b*) All *A. gracile* were removed by trapping from three 9-m² plywood pens situated in Marion Lake. Known numbers of animals were then placed in these pens and were allowed to settle down for about 2 weeks.

(*c*) On 10 nights during July and August 1969 a glass-bottomed boat was rowed along grid transects in the 1–2 m deep open-mud portion of the lake, which occupied 21% of the lake bottom. The transects were 1 m wide and had a mean length of 275 m. The total number of *A. gracile* seen per transect was recorded. On the nights when transects were run, records were made of the number of animals that could be seen in the plywood enclosures. This allowed a calculation of the percent *A. gracile* exposed at the time of sampling.

(*d*) The number of *A. gracile*/m² on the transects was calculated as follows:

$$D = \frac{N \times 100/P}{A} \qquad (1)$$

where D = density; N = number of animals seen on transect; P = percentage of animals observed in enclosures; and A = area of transect.

Correcting factors were employed to determine the density of *A. gracile* in subhabitats other than 1–2 m open mud. These factors were obtained as follows:

$$C = \frac{N_x}{N_m} \qquad (2)$$

where C = the correcting factor; N_x = the mean number of animals per trap in subhabitat *x* (Table 1); N_m = the mean number of animals per trap in the 1–2 m open-mud part of the lake.

It was assumed that the catchability of *A. gracile* did not vary between subhabitats.

The number of animals in each subhabitat could be determined by the following expression:

$$N_y = D \times C \times A_y \qquad (3)$$

where N_y = the estimated number of *A. gracile* in subhabitat *y*; D = estimated density of animals 1–2 m

TABLE 1. *Ambystoma gracile* population size data for neotenous adults and young of the 2nd year. The data were collected over 79 trapping days between June 3 and September 19, 1969. The mean percentage of animals hunting on a given night was 35.8% (range 21.0–52.4%). The mean number of animals seen on a transect through 1–2 m open mud was 0.425/m².

	Lily pads	*Potamogeton natans* & *Equisetum* sp.	Submerged vegetation		Open mud (*m*)				Entire lake
			0–2 m deep	2+ m deep	0–1	1–2	2–3	3+	
No. animals caught	618	216	204	63	203	239	106	116	1762
No. traps	23	10	10	8	12	25	13	13	114
Mean no./trap	26.9	21.6	20.1	7.9	16.9	9.6	8.2	6.4	15.5
±1 SE	10.8	7.4	6.1	4.5	8.0	6.3	4.2	3.7	
Correcting factor	2.79	2.25	2.09	0.82	1.76	1.00	0.85	0.67	1.61
Area of substratum (*m*²)	5,481	9,795	7,020	5,290	17,190	30,245	15,015	19,800	109,836
A. gracile/m²	1.19	0.96	0.89	0.35	0.75	0.43	0.36	0.29	0.56
Total no. *A. gracile*	6,500	9,364	6,234	1,846	12,858	12,854	5,420	5,643	60,719
Mean wt *A. gracile* (*g*)	6.5	7.1	6.7	6.7	6.1	6.6	7.2	7.2	6.7
Estimated biomass/m² (*g*)	7.7	6.8	6.0	2.4	4.6	2.8	2.6	2.1	3.7
Total biomass (*kg*)	42.3	67.0	42.1	12.5	79.0	85.6	38.9	41.4	408.5
% lake area	5.0	8.9	6.4	4.8	15.7	27.5	13.7	18.0	
% biomass	10.3	16.4	10.3	1.2	19.3	21.0	9.5	10.1	

mud; C = correcting factor; A_y = area of subhabitat y.

The total of these figures gives an indication of the total lake population, excluding young-of-the-year, which were not caught in funnel traps and were not seen on the transects. The only animals included in the estimate are larvae in their second summer and neotenous adults.

(*e*) Over the 1969 trapping period the mean number of *A. gracile* per trap was calculated for each substratum type.

Results

AMBYSTOMA GRACILE POPULATION

The calculated *A. gracile* population approximated 14,500 adults and 45,000 young of the 2nd year (Table 1, Fig. 2). This represents a total midsummer biomass of 408 kg.

Size class distribution

The size frequency distributions of *A. gracile* for various periods and areas revealed three main size classes within the population (Fig. 2).

Young-of-the-year — These animals were about 0.9 cm long when they hatched during May and June. Larvae were not recruited into the trapped population until September probably because of their small size and their tendency not to move around until their legs are fully developed at 2.5–3.5 cm.

In May 1970, during a *Rana aurora* egg count, an attempt was made to count *A. gracile* eggs. It was found that *Ambystoma* eggs were often difficult to see, but about 750 egg masses were discovered. Egg masses were found in all quadrats of the lake. Lindsey (1966) found that the mean number of eggs in 22 egg masses was 47.4. Reckoned on this basis, at least 35,000 larvae of *Ambystoma* entered the population in 1970, but this may be an underestimate since many egg masses were probably not found.

Young of the previous year — In June 1969 about 78% of the trapped animals were 1 year old. At this time the larvae entering their 2nd year were 1.5–5.5 cm in body length, with the highest proportion of them being between 4.0 and 4.5 cm long (Fig. 2). By August most of these animals were 4.5–5.0 cm long; much of the increase in mean population size during the summer was no doubt due to their growth (Fig. 2). In late August and early September a large proportion of the 2nd-year animals metamorphosed. The peak that they formed in the size distribution disappeared, and young-of-the-year formed a peak in the lower size ranges as they entered the trapped population.

Second-year animals formed almost the entire *A. gracile* population in 1 m deep areas with an open-mud bottom. In all subhabitats in the lake this age class was in the majority, although they were in relatively low proportion in deep areas and beds of *P. natans* and *Equisetum* sp.

Adults — Efford and Mathias (1969) have found that Marion Lake *A. gracile* must be at least 5.0 cm long before they possess gonads, but it appears

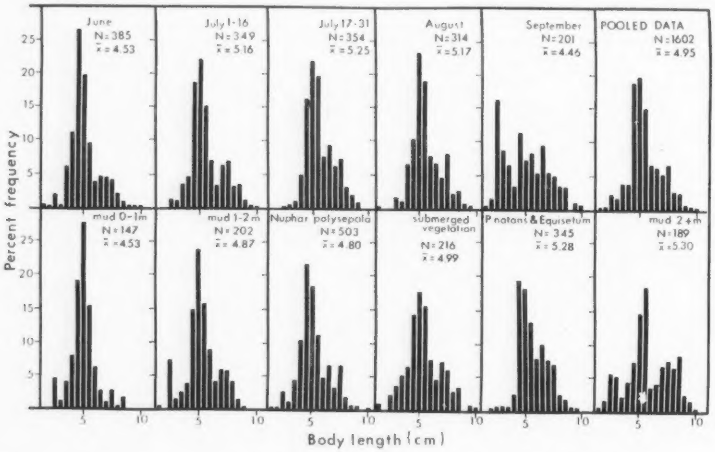

FIG. 2. *Ambystoma gracile* size frequency distributions plotted through time and between areas with different substrata.

that most animals mature at about 5.5–6.0 cm body length. This is also the size at which metamorphosis may occur. In June about 22% of the animals trapped were of adult size.

About 35% of the neotenous adult *A. gracile* caught were in open-mud areas deeper than 2 m. In these areas adults formed about 40% of the population. Few adults were caught in shallow open-mud areas.

Not all of the *A. gracile* adults breeding in Marion Lake were neotenous. In April 1969, on nine nights of observation, 106 metamorphosed and 117 neotenous adults were seen. These numbers were not significantly different from a 50:50 ratio (χ^2, $P = .01$). Only three breeding pairs were seen, all of which consisted of a metamorphosed male and a neotenous female. It was not known what proportion of the adult population bred during the year. All metamorphosed adults appeared to have left the lake by the 1st week in May.

TARICHA GRANULOSA POPULATION

The *T. granulosa* population was estimated at 2450 with the 95% confidence limits ranging from 2363 to 2542. The mean weight of adult *Taricha* was 7.5 g so the total lake biomass of this animal was about 18 kg. Little was discovered about the eggs and larvae of *T. granulosa*. This animal is known to lay its eggs singly or in small clumps (Chandler 1918), and females were seen laying eggs in this way in Marion Lake. The eggs were very small, however, and could not be found during diving excursions. Few *Taricha* larvae were found in the lake. These animals were too small to be caught in funnel traps frequently, and it is probable that many of those larvae trapped were erroneously identified as *A. gracile* larvae.

In early August 1969 the first *Taricha* larvae of the year were found in the lake. Four of the five larvae caught had a body length of 2.5–2.7 cm and were undergoing metamorphosis to the terrestrial stage when they were caught. One specimen caught on August 20 was 0.9 cm in length and had reached the stage of development where it had two toes on each hind foot.

The size frequency distributions of the male and female adult *Taricha* populations both approximated bell-shaped curves (Fig. 3). No seasonal changes in size class distribution could be recorded in either sex, since *Taricha* is a slow-growing animal. The mean growth rate of males is 0.93 mm/year and that of females is 0.59 mm/year (Efford and Mathias 1969). The sex ratio was not significantly different from 1:1 (*t*-test, $P = 0.05$) in the *Taricha* population as a whole and in individual centers of concentration. The size frequency distribution of *Taricha* appeared to be constant for the various areas as well as at different periods during the study period.

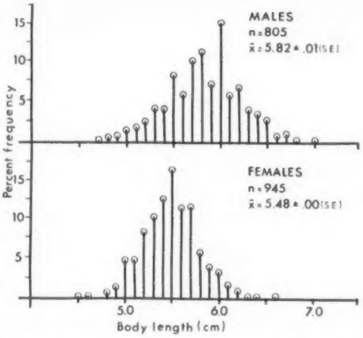

FIG. 3. *Taricha granulosa* adult size frequency distributions.

TRAPABILITY

Taricha granulosa were attracted to funnel traps. In the laboratory and in Marion Lake, newts would enter and leave traps in much the same way as they would enter and leave clumps of vegetation or fishnet. *Ambystoma gracile* did not appear to be attracted to traps and there were indications that some degree of trap-avoidance learning took place with these animals. Many trapped *Ambystoma* showed "fright" reactions such as violent swimming. This was not observed among trapped *Taricha*.

POPULATION DISTRIBUTION AND MOVEMENTS

Ambystoma gracile observed from a boat at night appeared to be widely dispersed. Animals seen to approach each other closely did not show agonistic behaviour, although when two animals were seen to touch, both animals would usually swim rapidly apart.

Although 504 *A. gracile* were individually marked, only 16 were recaptured. Since only five of these were caught more than 1 day after first capture, the returns were too scanty to allow any rigorous statement about how extensive individual *A. gracile* movements are. However, all recaptured animals were found in the subhabitat type that they were marked in. Furthermore, since the size compositions and trapped numbers of *Ambystoma* in individual subhabitat types did not change from early June to late August, it appeared that there were no large shifts in the *Ambystoma* population distribution except during the early spring, when metamorphosed adults entered the lake for a few weeks and during early September when metamorphosed young of the 2nd year left the lake.

The total weekly catch resulting from all methods of capture indicated that in 1969 the first *Taricha* entered Marion Lake around mid-April and the last animals left it in late September or early October (Fig. 4). There were two peaks in the catch per unit of effort–time curve, since the catch dropped off during late June and early July. During the time of peak catches amplexed pairs were common, but during the time of low catches no amplexed pairs were found.

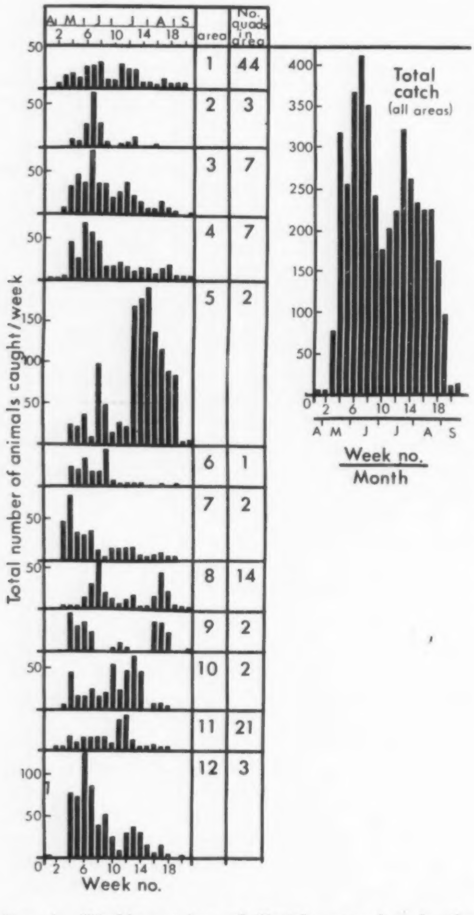

FIG. 4. Weekly catches of *Taricha granulosa* in the areas of the lake as a whole. The catches are the total result of trapping, diving, and netting from boats. During week 14 no diving was done. The probable diving catch was extrapolated from the numbers of animals caught by other methods.

The trapping data show that there is approximately a linear relation between the length of time since an animal was marked and the number of times that an animal was recaptured (Fig. 5). This suggests that many animals tended to remain in the lake throughout the season. However, the spatial distribution of the population within the lake changed through time (Fig. 4, Table 2).

The distribution of the *Taricha* catch was contagious. Large numbers of *Taricha* tended to form aggregations similar to those described by Farner and Kezer (1953) and Coates et al. (1970). In some instances up to 190 animals could be caught by hand in an area of about 15 m², as in area 5 during the 1st week in August. Although the location of concentration centers changed through time, they appeared to serve as a "home range" for the animals inhabiting them. Efford and Mathias (1969) found that of 315 animals they displaced, 314 returned to the site from which they were captured. A displacement experiment done by the author during July 1968 yielded similar results.

Thirty-five individuals were captured eight or more times during 1969. For each of these animals a list was made of the areas of the lake in which it was captured (Table 2). These animals all shifted their home range at least once. One animal was caught in seven of the 12 areas in the lake. The same pattern held true for animals caught fewer numbers of times. This and the stability of the population (Fig. 5) indicates that shifts in the sites of concentration centers observed during the 1969 season reflect the movements of individuals within the population and not the influx and egress of animals to and from the lake.

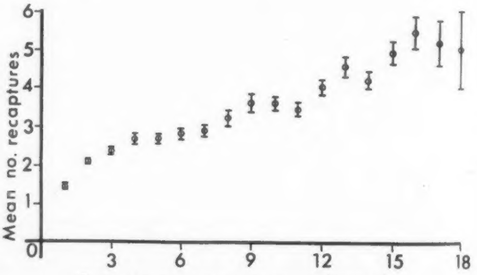

FIG. 5. A plot showing the relation between the time during which *Taricha granulosa* were marked and the mean number of times that they were recaptured. The vertical bars show standard errors.

TABLE 2. Areas of capture for *Taricha granulosa* caught eight or more times during 1969. The areas of capture are shown in chronological order.

Animal no.	Capture no. 1	2	3	4	5	6	7	8	9	10	11	Capture date (*first and last*)
1	7	7	7	5	5	5	5	5				14/5–1/9
2	7	12	7	12	5	5	5	5				21/5–19/8
3	12	12	4*a*	4*a*	12	12	5	5				21/5–26/8
4	12	12	7	12	12	1*b*	5	5				21/5–26/8
5	4*b*	6	7	6	4*b*	5	5	5				21/5–26/8
6	12	11	12	12	12	12	6	12				22/5–11/8
7	12	12	12	8	9	10	9	5				22/5–1/9
8	6	6	6	6	4*c*	6	6	10				23/5–18/7
9	1*b*	8	8	2	1*b*	3	5	5				26/5–12/8
10	12	12	12	10	10	10	10	10				28/5–18/8
11	4*b*	5	8	5	5	4*a*	5	5				2/6–1/9
12	6	5	6	4*c*	5	6	5	5				4/6–1/9
13	1*b*	2	11	12	7	5	8	3				12/6–27/8
14	12	10	9	12	5	5	11	12				14/6–21/8
15	2	3	3	3	3	3	3	3				18/6–21/7
16	7	7	7	6	5	5	5	5	5			14/5–26/8
17	7	4*b*	4*b*	5	9	6	7	5	5			14/5–26/8
18	7	4*c*	5	6	4*a*	5	5	5	5			21/5–26/8
19	4*b*	4*b*	6	4*a*	5	4*a*	5	5	5			21/5–1/9
20	12	12	12	12	5	5	10	5	5			21/5–1/9
21	3	3	3	8	5	5	5	5	5			22/5–5/9
22	9	10	9	12	11	12	11	10	9			23/5–18/7
23	12	12	12	12	11	10	10	10	10			23/5–24/7
24	12	1*b*	10	10	10	11	10	10	10			23/5–18/8
25	12	12	11	10	12	10	10	5	9			26/5–22/7
26	12	12	10	5	10	5	8	9	5			10/6–1/9
27	5	9	5	9	3	9	8	9	9	9		7/5–23/6
28	8	1*b*	12	7	12	10	10	11	10	10		14/5–6/8
29	7	5	2	3	5	9	5	5	5	5		15/5–19/5
30	9	9	12	12	12	11	12	12	5	8		20/5–15/8
31	12	12	12	7	11	12	12	3	1*b*	10		21/5–18/8
32	7	7	12	12	10	10	5	10	5	5		21/5–19/8
33	6	6	6	12	12	6	7	5	5	8		22/5–26/8
34	11	7	7	12	7	12	7	7	6	8	5	12/4–10/6
35	12	12	10	12	9	12	10	5	10	5	5	23/5–5/8

The placement of concentrations appeared to be correlated with the following factors.

Spatial complexity

All major centers of concentration were spatially complex. Areas 2, 4, 6, 7, 9, and 10 contained thick clumps of vegetation or tree roots. *Taricha* were also found in clumps of fishnet placed in the lake. The large numbers of animals caught in area 12 were almost all found clinging to a doubled layer of fishnet on a large pen built in the summer of 1967. Similarly, a piece of netting placed in area 10 during capture week 10 rapidly became a concentration center.

Temperature

In laboratory experiments *Taricha*, in water above 20 C, tended to lose their aquatic secondary sex characteristics and left the water if given the chance (Neish MS 1970). In the lake, as the surface water neared 20 C during late June, shallow water concentration centers disappeared and large numbers of animals were found in deeper areas where the temperature was about 15 C.

Social interaction

The presence of *Taricha* at a concentration center may have attracted other *Taricha*. During capture week 15, 30 newts were placed in a cylindrical cage of hardware cloth. A clump of ¾-inch fishnet was draped around this cage. Although no animals established themselves on the netting, a number of animals established themselves in a *P. natans* bed immediately adjacent to it, and when the cage was removed this concentration center ceased to exist. Twitty (1966) has shown that *Taricha torosa* males are attracted to females through the use of pheromones. In Marion Lake *T. granulosa* males were attracted to other males as well, at least to the point where homosexual amplexed pairs were occasionally seen. Few amplectic pairs were seen in clumps of *Taricha* in concentration centers, so these aggregations may not have been simply breeding aggregations.

Prey

During the 4th week of trapping, large numbers of *R. aurora* tadpoles hatched in areas 9 and 10. At this time *Taricha* appeared in these areas and were found to be eating tadpoles. As the tadpoles dispersed there was a decrease in the number of *Taricha* caught.

Mortality

Taricha granulosa was not eaten by the fish in Marion Lake, but *A. gracile* were found in the stomachs of rainbow trout (Efford and Mathias 1969), and one specimen was seen in the process of being eaten by a diving bug (*Lethocerus americanus* (Leidy)). The potent poison possessed by *Taricha* (Brodie 1968) makes it unlikely that newts were eaten by the mink, heron, or mergansers that periodically frequented the lake, although some *Ambystoma* may have been eaten by these animals. *Ambystoma gracile* in the process of regenerating limbs and tails were frequently captured in the lake, but this was never the case with *T. granulosa*. No dead *Taricha* were found in the lake although dead *Ambystoma* were occasionally found. These specimens were rapidly consumed by carrion feeders (such as tadpoles and amphipods) or were decomposed.

Discussion

The *T. granulosa* adult population size estimate made during the present study was similar to Efford and Mathias' (1969) estimate of 3965 individuals. In the present study a large proportion (about 83%) of the population was marked, so the estimate of 2450 individuals can probably be regarded with some confidence.

The *A. gracile* population estimate of Efford and Mathias (2300 individuals) differed radically from the present estimate of 14,500 adults and 45,000 2nd-year larvae. Efford and Mathias' estimate was based on the ratio of *T. granulosa* to *A. gracile* captured in funnel traps. Acceptance of such a method depended on the assumption that the two species were equally trappable. This assumption was not tenable, since *Taricha* appeared to be attracted to traps and *Ambystoma* to have avoided them to some extent. Avoidance learning has been demonstrated in *Ambystoma punctatum* (Schneider 1968), and the failure of Efford and Mathias to obtain consistent trapping records in areas repeatedly trapped to saturation may have been due to this phenomenon, as they suggested.

According to the present estimate there were about 6000 *A. gracile*/ha in Marion Lake, excluding young-of-the-year. This is high when compared to the densities of 41–3238/ha recorded among terrestrial adult salamanders (Anderson 1960; Hendrickson 1954; Stebbins 1954), but it must be remembered that the present census included 2nd-year larvae. There were about 1450 neotenous adult *A. gracile*/ha in the lake.

The large estimate of *A. gracile* numbers was not expected on the basis of trapping results and daytime observations. The nocturnal feeding habits of these animals and the short time during which they are exposed as they hunt (Neish MS 1970) make *Ambystoma* inconspicuous except during the early hours of darkness, when most salamander feeding occurred. Personal communications from researchers working in lakes in British Columbia and northern Manitoba suggest that neotenous *A. gracile* and *Ambystoma tigrinum* may be abundant in many lakes. Efford (1970) has estimated that *A. gracile* accounts for about 55% of the benthic predation by salamanders and fish in Marion Lake. This means that in Marion Lake *Ambystoma* is a significant predator.

Although *T. granulosa* is not nearly so abundant as *A. gracile* in Marion Lake, it may still have a significant effect as a predator since there was evidence that *Taricha* tends to concentrate its predation in small areas. The incursion of newts into tadpole hatching areas is an example of the response of *Taricha* to prey in high density. Such invasions may have a great effect on prey mortality (Neish MS 1970; G. Calef, personal communication); and predation behavior of *A. gracile* and *T. granulosa* is considered in detail in Neish (MS 1970).

It was estimated that in Marion Lake there were about 14,500 *A. gracile* of a size consistent with the possession of gonads, yet only about 750 egg masses were counted. The reasons for the discrepancy between the number of egg masses and the estimated number of adults could not be explained since little is known about breeding behavior of mixed neotenous–metamorphosed *A. gracile* populations. Calef (personal communication), in working on the breeding chorus of *R. aurora* in Marion Lake, has also found a low number of egg masses/adult population ratio. Anderson (1960) found a similar disparity in populations of the salamanders *Batrachoseps attenuatus* and *Aneides lugubris*, associated with high population density and resorption of eggs by females.

The distributions of *T. granulosa* and *A. gracile* were different, with the latter having a widely dispersed, apparently stable distribution, whereas the former had a highly contagious distribution that underwent drastic changes throughout the spring and summer. Dispersal of the *A. gracile* population may have been related to *Ambystoma*'s tendency to snap at each others' limbs and gills, which resulted in the two animals involved swimming away from each other. In the laboratory the loss of limbs and gills was fairly common. This was not the case with *T. granulosa*. Neither in the laboratory nor in the lake did *A. gracile* gather in closely knit masses similar to those commonly found among *T. granulosa*.

Coates et al. (1970) described a large concentration of *T. granulosa* in Clear Lake, Oregon, and concluded that the aggregation was postreproductive. Concentrations of animals in Marion Lake were seen during the entire study period, and amplectic pairs and egg-laying females were found both in and near centers of concentration, so they cannot be regarded simply as a postreproductive phenomenon.

An outstanding characteristic of the distribution of *T. granulosa* is that considerable changes were observed as the year progressed, both in the numbers of animals in the lake and in the location of concentration centers; no *Taricha* could be found before mid-April and after late September, and the catch per unit of effort decreased during late June and early July. At this time animals may have been wandering in and out of the lake (Pimentel 1961). Coincident with the decrease in the *Taricha* catch was an absence of amplectic pairs and a decrease in the volume of fish stomach contents observed by Sandercock (MS 1969) and a drop in macrophyte productivity observed by Davies (1970).

All concentration centers were found in complex areas such as tangled vegetation or fishnet. Changes in the locations of concentration centers appeared to be related to the effects of temperature and prey abundance. Shallow water concentrations disappeared as temperature rose during the late spring, and new concentrations formed in cooler, deeper areas. Depth alone probably did not restrict the distribution of *T. granulosa;* large concentrations have been found at depths up to 40 ft in Loon Lake, B.C.

Movements of individual *Taricha* suggest an appraisal of the term "home range" in connection with these animals. Displacement experiments of Efford and Mathias, as well as those done in the present study, indicate that *T. granulosa* rapidly returned to the point from which they were removed. It was evident that *T. granulosa* homed through water, unlike *T. torosa*, which homed over land (Twitty 1966). The home ranges to which the animals returned can at best be described as temporary, however, since some animals were caught in seven areas of the lake in 21 weeks, and most of the animals caught five or more times were captured in more than one area.

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DDT Residues in Canadian Atlantic Fishes and Shellfishes in 1967

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DDT residues were average or less than average in lobsters, five species of molluscan shellfishes, and seven species of finny fishes from estuarial and coastal waters of New Brunswick and Prince Edward Island, compared with the same or similar species elsewhere in North America and northern Europe. Mackerel had the highest residues, averaging 0.38 ppm of DDT or 0.54 ppm of DDT plus metabolites. These levels do not seem to be an immediate danger to the fish. In terms of human diet, residues in mackerel are as high as those in some beef. The other species averaged 0.1 ppm or less of total DDT residue in the whole body or muscle, usually much less. This is relatively low by present day findings. Viscera of salmon, cod, and hake had 6–15 times higher concentrations of DDT residues than did muscle. Flounder viscera had no more residue than did muscle, whereas lobster eggs had nine times as much as muscle. None of the four sampling locations produced consistently higher residues than the others.

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LITTLE seems to be known about insecticide residues in Canadian Atlantic coast fishes, except that Duffy and O'Connell (1968) found DDT residues in mackerel from the Gulf of St. Lawrence. The purpose of the present survey was to explore DDT concentrations in a greater variety of east coast species.

Materials and Methods

SAMPLE COLLECTION

Thirteen species were sampled from October 16–20, 1967. General locations are shown in Fig. 1; exact details are in a manuscript report (Sprague et al. MS 1969). Specimens were from regular commercial catches or could have been taken in commercial catches. Most samples were frozen within a few hours after being caught; cod, hake, and flounders were obtained in "fresh" iced condition and frozen within 1 day of being caught. Samples were frozen and held in domestic freezers for a maximum of 4 days after being collected. They were transported without thawing to the University of Prince Edward Island where they were placed in a deep freeze at –20 C and held until chemical analyses were completed in January 1968.

Three kinds of samples were used. For each kind, four or five duplicate samples from different individuals were analyzed. *Whole body*: the whole animal was frozen, and the entire blended sample, or an aliquot, used; for bivalve molluscs, the sample included all the soft parts inside the shell, but not the contained sea water or "liquor." *Muscle*: for fin fishes, a sample was taken from the back and flank; for lobsters, from the tail. *Viscera*: a sample comprised pieces taken from the major organs and tissues within the body cavity — intestine without contents, stomach, spleen, kidney, heart, gonad if present, and any fatty tissues connected to these organs; the amount of tissue taken was somewhat in proportion to the bulk of the organ.

ANALYTICAL METHODS

Measurements were made of the *p,p'* forms of DDT, DDD, and DDE. The purified extract from a portion of blended homogenate of the sample was analyzed by gas-liquid chromatography. Representative samples were confirmed on two gas chromatographic columns. The lower level of detectability was 0.01 ppm or lower, depending on the sensitivity of the column. Residues found in five samples were confirmed by thin-layer chromatography. Procedure details have been published (Duffy and O'Connell 1968).

Results are expressed in parts per million of tissue, wet weight. The term ΣDDT = ppm DDT + ppm DDD + ppm DDE. We did not multiply DDE by 1.11 to bring it to a molecular weight equivalent to DDT, as is sometimes done.

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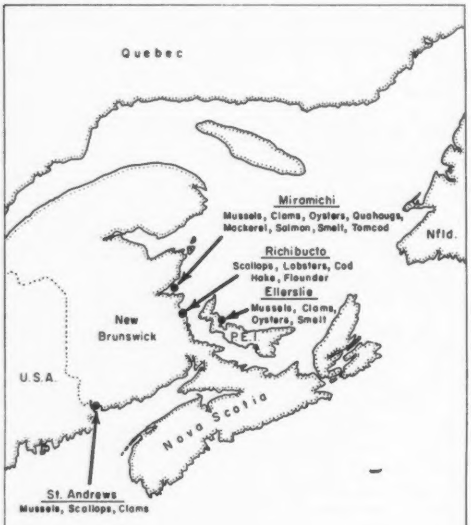
Quebec
Miramichi
Mussels, Clams, Oysters, Quahougs, Mackerel, Salmon, Smelt, Tomcod
Nfld.
Richibucto
Scallops, Lobsters, Cod Hake, Flounder
Ellerslie
Mussels, Clams, Oysters, Smelt
New Brunswick
P.E.I.
U.S.A.
Nova Scotia
St. Andrews
Mussels, Scallops, Clams
Atlantic Ocean

FIG. 1. Collecting points for marine fishes and shellfishes.

Results

Average results (Table 1) show that whole body residues were highest in mackerel, which had averages of 0.54 ppm ΣDDT and 0.38 ppm DDT. These averages adequately represent individual measurements, which were of the same order of magnitude in all five individuals. (For individual animal values, see Sprague et al. MS 1969, available on request from the St. Andrews Biological Station.) Of the other seven species for which whole body concentrations were measured, all had ΣDDT of 0.1 ppm or less. Within this low range, mussels were the highest of the molluscs; the other four molluscs that are more commonly eaten had extremely low residues. Total residues in muscle were low in the five species tested, and DDT itself was even lower. Not all individuals had measurable DDT; for example only one of the four lobsters did. Salmon, cod, and hake viscera averaged about 6, 15, and 10 times higher concentrations of total residue, respectively, than did muscle. However, flounders had no higher concentrations of residue in viscera than in muscle. In lobster eggs, concentrations of total DDT residues, mostly DDE, were higher than in lobster muscle.

The metabolite DDE was the commonest residue. It usually made up at least half, and often almost all, of the total residues. The exceptions were mackerel, in which most of the residue was DDT, and viscera of cod and hake, which had about as much DDT as DDE. Little DDD was found, except for small amounts in those samples that showed appreciable levels of DDT. No *o,p′*-DDT was detected in these samples, only *p,p′*.

Discussion

BIOLOGICAL SIGNIFICANCE FOR THE ANIMALS

For the most part, there seems no cause for immediate alarm for the welfare of these marine animals. From extensive studies, Butler (1966) considers that 0.01–0.02 ppm of DDT residues in oysters is "of negligible significance." Sixteen of the 20 samples we measured for whole body or muscle residue had 0.05 ppm ΣDDT or less, comparable with the harmless levels mentioned by Butler. Slightly higher residues were found in mussels from two of three locations, and in smelt from one of two locations.

Only mackerel were notably higher, averaging 0.54 ppm ΣDDT. This seems typical of the species. Duffy and O'Connell (1968) found about the same levels of DDT, although lower values for DDE, in mackerel from another part of the Gulf of St. Lawrence near Prince Edward Island. Mortality would not be expected even in mackerel. Mortality in fish is generally associated with a DDT content at least 10 times higher, commonly 4–7 ppm DDT in the whole body, or 3–12 ppm in the muscle, according to a recent summarization of available information (Sprague et al. 1971).

Effects on reproduction seem to be the greatest danger of residues. Developing salmonid fry may suffer mortality if there is 4.75 ppm DDT in eggs. This may result if there is at least 3.4 ppm in parent fish in laboratory tests (Macek 1968) or much more in wild fish (Burdick et al. 1964; Anderson and Everhart 1966). DDT level in mackerel is only about one-tenth of the danger level found by Macek. Residues in mackerel eggs should be investigated, but they may be no higher than in the general body tissues, which are also oily. In fact, mackerel roe has been found to have a little less residue than muscle (Duffy and O'Connell 1968).

SIGNIFICANCE AS HUMAN FOOD

At the time of writing, there are no official tolerances for DDT in seafood. Tolerances for meat are based on levels in the fat, so are not directly comparable. Mackerel would be the species

TABLE 1. **DDT residues measured in Atlantic marine fishes and shellfishes collected in October 1967. Averages of four or five samples from different individuals are given, followed by the range in parentheses. All values have been rounded to two decimal places. A dash (–) indicates less than 0.01 ppm. Average size of individuals is given in centimeters, with carapace length for lobsters.**

Species	Location[a]	Size (*cm*)	Organ	ppm			
				DDT	DDD	DDE	ΣDDT
Mussel, edible *Mytilus edulis* L.	M	7.6	Whole	–	–	0.05 (0.03,0.11)	0.05 (0.03,0.11)
Mussel, edible	E	6.7	Whole	0.01 (–,0.05)	–	0.06 (–,0.13)	0.08 (–,0.17)
Mussel, edible	St.A	4.4	Whole	0.04 (–,0.10)	–	0.05 (0.03,0.10)	0.09 (0.06,0.15)
Scallop, giant *Placopecten magellanicus* (Gmelin)	R	8.0	Whole	0.01 (–,0.07)	–	0.02 (0.01,0.04)	0.03 (0.01,0.09)
Scallop, giant	St.A	10.7	Whole	–	–	0.03 (0.01,0.03)	0.03 (0.01,0.03)
Clam, soft-shelled *Mya arenaria* L.	M	6.3	Whole	–	–	0.01 (–,0.04)	0.01 (–,0.04)
Clam, soft-shelled	E	6.7	Whole	–	–	0.05 (–,0.14)	0.05 (–,0.14)
Clam, soft-shelled	St.A	5.5	Whole	–	–	–	–
Oysters, eastern *Crassostrea virginica* Gmelin	M	7.8	Whole	–	–	0.01 (–,0.03)	0.01 (–,0.03)
Oysters, eastern	E	13.9	Whole	–	–	0.02 (0.01,0.02)	0.02 (0.01,0.02)
Quahaugs, *Venus mercenaria* L.	M	6.1	Whole	0.04 (–,0.21)	–	0.01 (0.01,0.02)	0.05 (0.01,0.23)
Lobster, American *Homarus americanus* Milne-Edwards	R	8.5	Muscle	0.01 (–,0.02)	0.01 (–,0.02)	0.03 (0.02,0.04)	0.04 (0.02,0.08)
Lobster, American	R	8.5	Eggs	0.04 (–,0.09)	0.01 (–,0.04)	0.31 (0.07,0.94)	0.36 (0.07,0.94)
Mackerel, Atlantic *Scomber scombrus* L.	M	41.8	Whole	0.38 (0.28,0.48)	0.08 (0.04,0.13)	0.09 (0.05,0.16)	0.54 (0.45,0.77)
Salmon, Atlantic *Salmo salar* L.	M	59	Muscle	0.02 (–,0.04)	0.01 (–,0.03)	0.03 (0.02,0.04)	0.05 (0.02,0.10)
Salmon, Atlantic	M	59	Viscera	0.08 (0.04,0.19)	0.06 (–,0.19)	0.16 (0.08,0.27)	0.29 (0.15,0.65)
Cod, Atlantic *Gadus morhua* L	R	58	Muscle	0.01 (–,0.04)	–	0.04 (0.02,0.05)	0.05 (0.02,0.08)
Cod, Atlantic	R	58	Viscera	0.40 (0.09,0.68)	0.10 (0.02,0.17)	0.24 (0.14,0.34)	0.75 (0.36,1.16)
Hake, white *Urophycis tenuis* (Mitchill)	R	64	Muscle	0.01 (–,0.03)	–	0.03 (0.01,0.05)	0.04 (0.01,0.08)

(*Continued*)

TABLE 1. DDT residues measured in Atlantic marine fishes and shellfishes collected in October 1967. Averages of four or five samples from different individuals are given, followed by the range in parentheses. All values have been rounded to two decimal places. A dash (–) indicates less than 0.01 ppm. Average size of individuals is given in centimeters, with carapace length for lobsters. (*Concluded*)

Species	Location[a]	Size (*cm*)	Organ	ppm			
				DDT	DDD	DDE	ΣDDT
Hake, white	R	64	Viscera	0.19 (0.08,0.36)	0.10 (0.04,0.16)	0.13 (0.05,0.24)	0.43 (0.17,0.76)
Smelt, American *Osmerus mordax* (Mitchill)	M	16.5	Whole	–	0.01 (–,0.03)	0.01 (–,0.04)	0.03 (–,0.06)
Smelt, American	E	19.4	Whole	0.06 (–,0.16)	–	0.05 (0.02,0.09)	0.10 (0.02,0.19)
Flounder, winter *Pseudopleuronectes americanus* (Walbaum)	R	28	Muscle	–	–	0.01 (–,0.03)	0.01 (–,0.03)
Flounder, winter	R	28	Viscera	–	–	0.01 (–,0.02)	0.01 (–,0.02)
Tomcod, Atlantic *Microgadus tomcod* (Walbaum)	M	24.5	Whole	–	–	–	–

[a] M, Miramichi; E, Ellerslie; St.A., St. Andrews; R, Richibucto.

of concern. However, the amount of ΣDDT in mackerel (average 0.54 ppm) is about the same as the higher of two averages reported for beef (0.14 and 0.5 ppm DDT + DDE) from the USA by Campbell et al. (1965). They estimated 0.20 ppm DDT + DDE as an average for meat, seafood, and eggs in a human diet in the USA. Canadian mackerel thus seems to be as acceptable as other animal food products.

Lower levels of residue were present in all the other fishes that we measured. They are comparable, not with high residues in the meat component of a human diet, but with lower average residues in total diet, estimated for the USA as 0.08 ppm DDT + DDE (Campbell et al. 1965), and 0.02 ppm of all chlorinated organic chemicals (Duggan and Dawson 1967).

GEOGRAPHIC VARIATION

There is no firm evidence of heavier DDT pollution in any one of our sampling locations. The comparison is based on smelt and four species of molluscs collected from two or more locations, and is incomplete, but any major difference should have shown. Residues from Ellerslie samples were not consistently higher despite heavy use of agricultural chemicals in P.E.I. Nor were higher residues found in samples from the Miramichi estuary, although the river drains a forested area of New Brunswick extensively sprayed with DDT over the preceding 15 years. The year of sampling, 1967, was about the end of the DDT era in that large forest-spraying operation. That program apparently did not cause severe accumulation of DDT residues in marine fishes, or this would have shown in our survey.

Comparison with similar species elsewhere shows that Atlantic Canadian sea fishes are average or low in DDT residue. For example, our average values for 11 samples of molluscan shellfishes had a median of only 0.03 ppm ΣDDT, comparable with 0.02 ppm or less that Butler (1968) considers "as low as we find anywhere in the nation," and somewhat lower than the median of 0.05 ppm ΣDDT reported by Campbell et al. (1965) for shellfishes from eight U.S. states. Other findings from North America and northern Europe indicate that Canadian Atlantic molluscs, cod, and hake are in the low part of the reported range of DDT residues, and lobsters and flounders have residues as low as those reported anywhere for marine crustaceans and marine flatfish respectively (documentation is provided in our manuscript report). Miramichi salmon are low in residue compared with the high levels of 3.4 ppm ΣDDT in Baltic salmon (Jensen et al. 1969) or 0.6 ppm ΣDDT in king salmon from California (Keith and Hunt 1966).

The Canadian salmon are similar to sea trout from a British estuary, which have about 0.03 ppm ΣDDT (Holden 1966).

Somewhat elevated levels of residue in Canadian mackerel may have been partly because of the oiliness of the fish, DDT being stored in fatty tissues. However, the higher levels may also be the result of geographical variation. Mackerel is the only species examined that is not native to the area. It is a warmwater migratory fish, visiting the Gulf of St. Lawrence in the summer. Its center of distribution is southward from the Gulf of Maine and it may be found close inshore along the New England and middle Atlantic states (S. N. Tibbo, personal communication). Therefore, our mackerel sample may reflect higher levels of residue in fish from more southern parts of the coast in the USA. Butler (1969) confirms that oysters from Maine, adjacent to New Brunswick, had little contamination with DDT, but oysters from more southerly Atlantic states were more contaminated.

Scope for Additional Study

Food-chain accumulation has not been adequately covered in this survey. Future studies should include animals such as fish-eating birds and seals. Atlantic Canadian seals have been found to have high levels of DDT residues in their blubber (Holden and Marsden 1967).

DDT residues should be measured in mackerel eggs from several places, and also in lobster eggs, since crustaceans are known to be sensitive to chlorinated hydrocarbons.

The low levels of DDT that we found in Canadian shellfishes would not necessarily prevail all the time. Studies in USA (Butler 1966) and Britain (J. E. Portmann, personal communication) indicate wide biweekly fluctuations in oysters, apparently reflecting short-term changes in the environment.

We have no immediate explanation for the absence of detectable residue in tomcod. The low residues in flounders compared to cod and hake from the same location may result from a seasonal decrease, as has been found for flounders in Massachusetts (Smith and Cole 1970).

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Population Biology of Lake Trout (*Salvelinus namaycush*) of Lake Superior before 1950[1]

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Scale samples collected in 1948 were used to estimate the instantaneous total mortality rate (0.70) and growth for lake trout (*Salvelinus namaycush*) in Lake Superior before the population had been significantly reduced by the sea lamprey (*Petromyzon marinus*). Indirect evidence indicates that the instantaneous natural mortality rate was probably 0.10–0.25. The Ricker model was used to calculate yield per recruitment, which varied with natural mortality and growth. Natural mortality was more critical than growth; yield per recruitment increased 183.3% with a 60% decrease in instantaneous natural mortality (from 0.25 to 0.10). For the prelamprey lake trout population the yield per recruitment was about 12–34 lb; the recruitment of about 3.6–10.1 million lake trout of age 1.5 resulted in an annual commercial production of 4 million lb.

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DURING the 1950's, while the abundance of sea lampreys (*Petromyzon marinus*) was increasing rapidly in Lake Superior, the commercial production of lake trout (*Salvelinus namaycush*) was declining drastically; it dropped from about 4.7 million lb (dressed weight) in 1950 to about 0.5 million lb in 1960 (Baldwin and Saalfeld 1962; Hile et al. 1951; Smith 1968). Consequently, in 1956 the United States and Canadian governments initiated a cooperative program of sea lamprey control and lake trout rehabilitation. Millions of dollars have been spent on this program. The cost of lamprey control alone in Lake Superior was about $410,000 in 1968 (Brinser et al. 1968); in addition, more than 3.4 million yearling lake trout were stocked in that year (Great Lakes Fishery Commission 1969).

Recently the lake trout stocks have shown signs of recovery. The abundance index in the State of Wisconsin waters in 1968 was more than four times that in 1961, and significant numbers of native juvenile trout were present (Great Lakes Fishery Commission 1969). The present lake trout population is not, however, entirely comparable to the prelamprey population (i.e., the population before 1950, when the effects of lamprey predation were still negligible and the annual commercial production of lake trout was about 4 million lb); most of the present stocks are hatchery-released fish and lamprey predation is a significant source of natural mortality. Nevertheless, information on the prelamprey lake trout population is of value as an indicator of possible future returns from lamprey control and lake trout rehabilitation.

Approximate estimates of mortality rates, growth, and yield per recruitment are presented here for lake trout in the prelamprey population.

Estimate of Total Mortality

In June 1948, scale samples were collected by the U.S. Fish and Wildlife Service from commercial gillnets (4.5-inch mesh, stretched measure) and set hooks at five locations between the Keweenaw Peninsula and Munising, Michigan. We believe that these samples (Table 1) represent the only sizable collection of scales from the prelamprey lake trout population of Lake Superior.

Although the samples came from a relatively small geographical area of the lake, we consider them to be representative of the lakewide population of typical "lean" lake trout (other than siscowets and "humpers" — see Eschmeyer and Phillips 1965). Eschmeyer (1957a) found evidence of lake trout subpopulations in Lake Superior, but tagging

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TABLE 1. Age distribution of Lake Superior lake trout collected between the Keweenaw Peninsula and Munising, Michigan, 1948.

	No. fish		
Age-group	4.5-inch mesh gillnet	Floating set hooks	Total
III	5	—	5
IV	18	—	18
V	21	—	21
VI	10	2	12
VII	45	7	52
VIII	109	37	146
IX	95	54	149
X	63	45	108
XI	42	53	95
XII	25	31	56
XIII	13	10	23
XIV	4	4	8
XV	—	5	5
XVI	—	1	1
Total	450	249	699
Avg age	8.7	10.1	9.2

of native lake trout on the spawning grounds by Eschmeyer et al. (1953) and Rahrer (1968) showed that large lake trout (20–30 inches long) move extensively throughout the lake and segregate only during the fall spawning season. Smith and Van Oosten (1940) found that tagged native lake trout ranged widely in Lake Michigan, and Pycha et al. (1965) described extensive movements for hatchery-released lake trout in Lake Superior. These studies indicate that, although subpopulations exist, they mix during the nonspawning period.

Catch curves were used to estimate total mortality (Ricker 1958), based on linear regression analysis and the slope of the right-hand limb. The instantaneous total mortality rate, Z, for lake trout caught in gillnets is 0.53 and on set hooks, 0.75 (Fig. 1). Covariance analysis indicated that the separate slopes were not significantly different from a common slope at $P = 5\%$ ($F_{1,9} = 3.885$), although set hooks caught predominantly older fish. The samples were, therefore, pooled, and a pooled Z of 0.70 (SD = 0.007) for ages IX and older was calculated (Fig. 2).

Our data do not satisfy all of the conditions necessary to ensure the validity of the catch curve (Ricker 1958): the samples were all obtained in 1 year and from 4.5-inch mesh gillnets and set hooks, whereas the fishery employed several other types of fishing gear (e.g., gillnets with meshes as large as 7 inches, trolling gear, and poundnets).

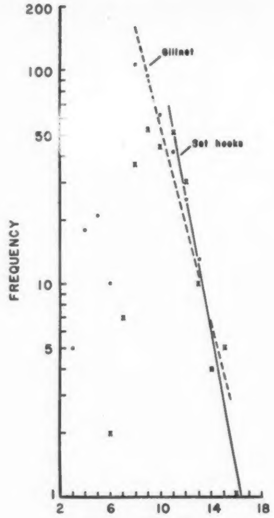

FIG. 1. Catch curves for lake trout of Lake Superior caught in gillnets (●) and set hooks (×). The slope of the line gives the instantaneous total mortality rate, Z (0.53 for gillnets and 0.75 for set hooks).

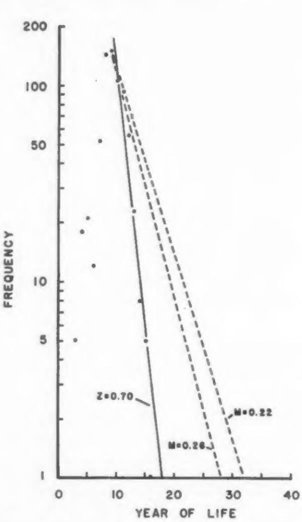

FIG. 2. Catch curve for lake trout of Lake Superior, all gears combined (solid line). The slope, or instantaneous total mortality rate, of the solid line is 0.70. The slopes of the broken lines give the instantaneous natural mortality rates, 0.26 and 0.22, for life expectancies of 28 and 32 years, respectively.

We believe, however, that because the data were from the predominant gears of the fishery and because the effects of fluctuations in year-class strength and mortality are small for the long-lived lake trout (Kennedy 1954), our estimate is a fair approximation for the prelamprey lake trout population.

Estimate of Natural Mortality

Natural mortality was estimated by a method in which constant recruitment and an exponential decrease in numbers with age are assumed. The survival of one fish from a cohort of 149 fish at age 9 to a life expectancy age was calculated. The instantaneous natural mortality rate, M, was then derived.

Measurements of tagged lake trout at release and recapture have suggested a growth rate of ½ inch or less per year for fish over 36 inches long (Eschmeyer 1957b). Rahrer (1967), on the other hand, reported a growth rate of 2–3 inches per year for large trout that were caught in 1953. Our data for large lake trout also suggest a growth rate of about 2 inches per year from a length of 35 inches at age 15 (five fish) to 37 inches at age 16 (one fish). Since Eschmeyer's estimate was based on only a few fish (P. H. Eschmeyer, personal communication) and these fish could have been adversely affected by the tag, and since most of Rahrer's fish were alive in 1948, we believe that the growth rate for fish over 36 inches long was about 2 inches or less per year during the prelamprey period.

Lake trout weighing 88 and 125 lb, about 61 and 69 inches long, respectively, have been reported caught in Lake Superior, although fish larger than 50 lb were uncommon (Van Oosten 1944). Although the lengths or weights of lake trout cannot be used to determine exact age, they can be used to estimate approximate age. Using 37 inches as the length at age 16 and a growth rate of 2 inches per year, we calculated the approximate age of a 61-inch fish as 28 years and that of a 69-inch fish as 32 years. Since a constant growth increment was used and growth in length at older ages is generally asymptotic (Beverton and Holt 1959), these ages are probably underestimates.

A reduction of a cohort of 149 lake trout at age 9 to a single individual at a life expectancy of 28 years corresponds to an M of 0.26; for a life expectancy of 32 years the corresponding M is 0.22 (Fig. 2). These mortality rates are, of course, overestimates if the ages are underestimates.

The natural mortality of lake trout in the Great Lakes has been small when lamprey predation was low (Table 2). Fry (1953) reported an M value of about 0.25 for lake trout of South Bay, Lake Huron, just before heavy lamprey predation began, and Pycha and King (1967) reported an M value of about 0.23 for lake trout that were stocked in Lake Superior in 1955 and recovered at age V. An M of 0.23 was also recorded for age III, hatchery-released fish of the 1954 year-class in South Bay, Lake Huron, by Budd et al. (1969). Silliman (1969a) obtained a reasonable fit to the lake trout catch in Lake Michigan before lamprey predation

Table 2. Mortality estimates for lake trout from various lakes.

Lake	Stock	Age-groups	Instantaneous mortality rate			Source
			Fishing	Natural	Total	
Cayuga, N.Y.	Hatchery	III+	0.14	0.63[a]	0.77	Webster et al. (1959)
Cold Stream Pond, Me.	Native	VI–IX	0.52	0.33	0.85	DeRoche and Bond (1957)
Great Slave, Ont.	"	–	0	0.60	0.60	Beverton and Holt (1959)
Great Slave, Ont.	"	X–XX	0	0.40–0.67	0.40–0.67	Kennedy (1954)
Huron, Ont.	Hatchery	III	–	0.23[a]	–	Budd et al. (1969)
		II–V	–	0.19–0.58[a]	–	
Huron, Ont.	Native	–	–	0.25[a]	–	Fry (1953)
		–	–	1.20[a]	–	
Manitou, Ont.	Hatchery	VII–XI	0.05	0.24	0.29	Budd et al. (1968)
Michigan	Native	–	0.50	0.20	0.70	Silliman (1969a)
Opeongo, Ont.	"	IX–X	–	0.34	–	Paloheimo (1961)
Opeongo, Ont.	"	IX–XI	0.57–0.58	0.25–0.32	0.82–0.90	Ricker (1958)
Superior, Ont.	"	VII–XI	0.01–0.02	2.30–2.29[a]	2.31	Loftus (1958)
Superior, Wis.	Hatchery	I–V	–	0.23[a]	–	Pycha and King (1967)
Superior	Native	IX+	0.45–0.60	0.10–0.25	0.70	Present study

[a] Sea lampreys were present when these estimates were made and probably contributed to the high values.

by using a simple population model with F = 0.50 and M = 0.20, and simulating the fishery on an analog computer. Other studies in smaller lakes show a wide range of natural mortality (Table 2). The evidence suggests that M was small, probably ranging from 0.10 to 0.25 for the prelamprey lake trout population of Lake Superior.

Estimate of Growth

To calculate growth in dressed weight (Fig. 3), average lengths at capture for each age-group in the 1948 samples were converted to dressed weights by the equation:

$$\log W_d = -3.636 + 3.062 \log L$$

where W_d is dressed weight in pounds and L is length in inches. Weights for fish younger than age III, which were not represented in the samples, were computed from average lengths given by Eschmeyer (1956).

There are several sources of bias in our estimate of growth — e.g., the method of calculation (Rahrer 1967) and the season and depth of capture (Dryer 1966; Dryer and King 1968). We believe, however, that the biases are small.

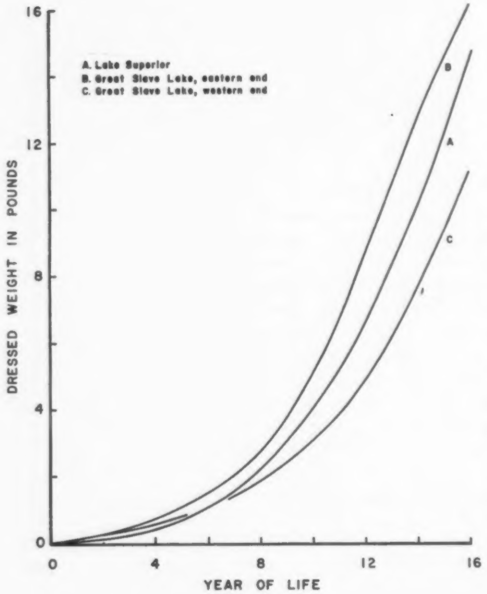

FIG. 3. Relation of dressed weight to age of lake trout of Lake Superior and Great Slave Lake. Great Slave Lake data from Kennedy (1954).

It is generally accepted that growth in most fishes is asymptotic with age, following an S-shape curve (Beverton and Holt 1959). Silliman (1969b) fitted the Gompertz growth equation to lake trout of Lake Michigan, and Beverton and Holt (1959) fitted the von Bertalanffy growth equation to lake trout of Great Slave Lake. Growth for the prelamprey lake trout, however, was nearly exponential with age (Fig. 3). This discrepancy is explained by noting that Lake Superior lake trout of age 16 are relatively young fish. The von Bertalanffy growth equation has an inflection at about one-third the asymptotic weight (Beverton and Holt 1959). When 100 lb is used as the asymptotic weight for the prelamprey lake trout, the inflection is at 28 lb dressed weight or at an age of approximately 20 years. Hence, the growth for the prelamprey lake trout of Lake Superior is in agreement with an S-shape growth relation.

Yield per Recruitment

Yield per recruitment was calculated by the Ricker model (Ricker 1958),

$$Y_e = \sum_{x=t_c}^{t_\lambda} F_x N_x w_x \frac{\exp(g_x - M_x - F_x) - 1}{g_x - M_x - F_x}$$

where Y_e = equilibrium yield per pound of recruits; x = a subscript specifying successive ages or periods in the life of the fish; t_c = age of first capture; t_λ = last or maximum age of capture; N_x = number of fish of age x; w_x = weight of fish of age x; g_x = instantaneous growth rate for age x; M_x = instantaneous natural mortality rate for age x; and F_x = instantaneous fishing mortality rate for age x. This model was selected because it uses a flexible time interval and no fixed growth relation. Main assumptions of the model are: (1) the population is stable, (2) growth and mortality rates are independent of population size, and (3) catchability is constant.

For our calculations we divided the year into two 6-month periods and set the growing season from July to December, since Lawrie (1962) and Rahrer (1967) found that most growth occurred during late summer and fall. Fishing mortality, on the other hand, was divided evenly between the two periods, since fishing effort was similarly distributed. Natural mortality was assumed to be evenly divided between the two periods.

We made several computer runs, using Paulik and Bayliff's (1967) program and one written by the senior author for the Ricker model, with different combinations of growth, fishing and natural mortalities, and age of first capture.

The first set of runs was made with the 1948 growth; recruitment at age 1.5 (0.033 lb); t_c at 6.5 years, which is the age at minimum size limit ($W_d = 1.4$ lb); and various values of instantaneous fishing mortality rate, F, with M of 0.10, 0.20, and 0.25 (Fig. 4). With Z (= total mortality; sum of fishing and natural mortalities) constant at 0.70, the yield per recruitment for M of 0.10 is 34 lb; for M of 0.20, 17 lb; and for M of 0.25, 12 lb. The difference in yield per recruitment for M of 0.25 and 0.10 is an increase of 22 lb or 183.3%, which is a considerable increase for a decrease in M of only 60%.

Tester (1953) found that the shape of the growth curve affected yield per recruitment in the Ricker model. To test this effect, we selected the growth of lake trout of Great Slave Lake (Kennedy 1954) for comparison. This selection was based on considerations such as species composition, size and location of the lake, and number of year-classes, which we felt produced growth similar to that for the prelamprey lake trout population of Lake Superior.

Kennedy (1954) gave two growth curves — one for the eastern end of the lake and one for the western end. These curves were converted to growth in dressed weight, and restricted to ages 1–16 (Fig. 3).

A second set of runs with M of 0.20, recruitment at age 1.5, t_c at 6.5 years, and F values from 0.01 to 0.80 was made with each growth pattern. The yield per recruitment for the large fish of eastern Great Slave Lake is 5.9% (1 lb) greater, and that for the smaller fish of western Great Slave Lake is 29.4% (15 lb) less than the yield per recruitment for lake trout of Lake Superior with Z of 0.70 (Fig. 5). It thus appears that the shape of the growth curve is not so critical as natural mortality in determining yield per recruitment for the prelamprey lake trout population.

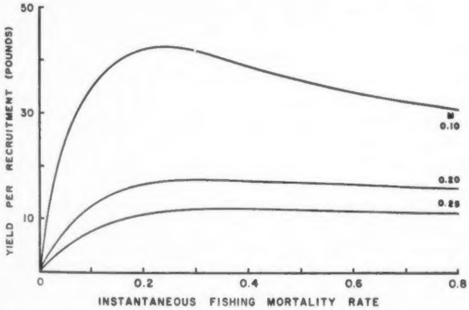

FIG. 4. Yield per recruitment for Lake Superior lake trout at different rates of fishing mortality with natural mortality (M) of 0.10, 0.20, and 0.25 (recruitment at age 1.5, 1948 growth, and age of first capture at 6.5 years).

It is possible, however, that a substantial change in growth, such as that reported for Lake Superior lake trout before the maximum abundance of sea lampreys by Rahrer (1967), may critically affect yield per recruitment. To test this point, we made another run, using Rahrer's growth data for fast-growing lake trout caught in 1953. Since Rahrer's data were reported in round weights and only for ages 1–12, they were converted to dressed weights and extrapolated beyond age 12 to obtain estimates for ages 13–16 (Fig. 6).

The yield per recruitment is 24 lb (for Z = 0.70), which is greater (41.2%) than the 17 lb obtained with slower growing lake trout caught in 1948. When t_c is reduced to 6 years, to adjust for faster growth in attaining the age at minimum size limit for fish caught in 1953, the yield per recruitment is slightly less, 22 lb. We can conclude, then, that a substantial increase in growth, such as that in 1953 (probably from the effects of lamprey predation), can affect yield per recruitment.

The effect of varying minimum age (size) and fishing mortality was investigated in the third set of runs; age of recruitment was fixed at 1.5 years, M at 0.20, and growth at the 1948 rate (Fig. 7). For a constant F, yield per recruitment can be increased by increasing the legal minimum age. When F is 0.50, only 17 lb of adult lake trout per pound of recruits are available when the minimum age of recruitment is 6.5 years. On the other

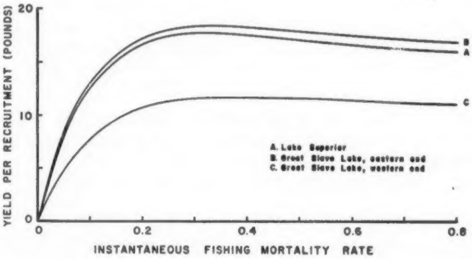

FIG. 5. Yield per recruitment for different rates of fishing mortality and growth of lake trout of Lake Superior and Great Slave Lake (recruitment at age 1.5, age of first capture at 6.5 years, and instantaneous natural mortality rate of 0.20). Great Slave Lake data from Kennedy (1954).

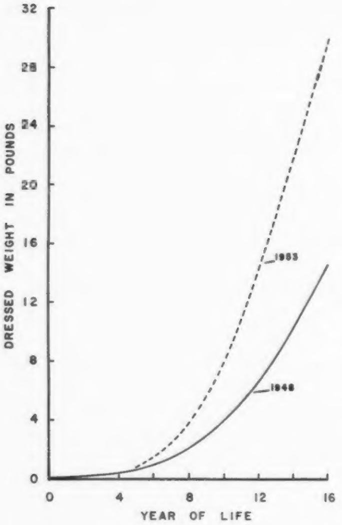

FIG. 6. Relation of dressed weight to age of lake trout of Lake Superior, 1948 and 1953.

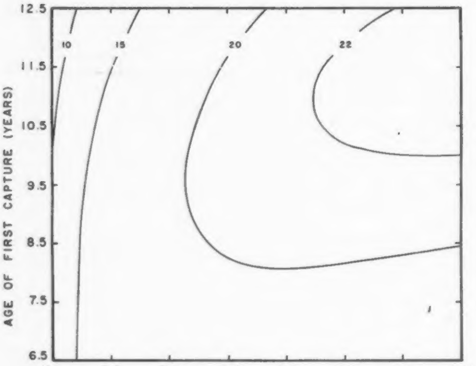

FIG. 7. Yield per recruitment for Lake Superior lake trout at different rates of fishing mortality and at various ages of first capture (recruitment at age 1.5, 1948 growht, and instantaneous natural mortality of 0.20).

hand, when this minimum age is increased to 10 years, the yield is 22 lb of adult lake trout per pound of recruits, an increase of 29.4%.

Increasing both fishing mortality and the minimum age result in even greater yields per recruitment than if only one of the parameters is increased. For example, the yield per recruitment with F of 0.70 and t_c at 11.5 years is about 23 lb, an increase of 35.3% over the 17 lb obtained with F of 0.50 and t_c at age 6.5.

Small changes in any of the parameters — growth, fishing and natural mortalities, and age of first capture — probably do not produce disequilibrium within the population and violate the assumptions of the Ricker model. Large changes, on the other hand, have profound effects on the population, which are not accounted for in the model. Hence, extrapolating beyond conditions that existed during the prelamprey period is questionable.

Indirect evidence indicates that the prelamprey lake trout population was in a steady state. Annual production was fairly stable at about 4 million lb, and the catch per unit of effort was extremely stable, especially before 1945 (Hile et al. 1951). Yield per recruitment from the Ricker model was probably between 12 and 34 lb, depending on natural mortality and growth. Recruitment to sustain the annual production of 4 million lb was, therefore, about 3.6–10.1 million lake trout of age 1.5 — or considerably more than are provided at the present annual stocking rate of about 3 million yearlings (Great Lakes Fishery Commission 1969).

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Ecological Energetics and Natural History of *Hedriodiscus truquii* (Diptera) in Two Thermal Spring Communities

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The interrelations of flora and fauna of the Ohanapecosh Hot Springs, Mount Rainier National Park, Washington State, were studied during a 4-year period commencing in 1963. The flow of energy and efficiency of its transfer were measured in two springs, one with an annual mean temperature of 35 C (SD 2.0) and the other a constant 47 C. In addition to temperature differences, each spring contained a different flora and fauna.

Primary production estimates were obtained together with data relative to many aspects of the natural history of the dominant dipteran herbivore *Hedriodiscus truquii* (Bellardi), hitherto undescribed in the larval stage, and common to both springs. Aspects of the natural history of *Hedriodiscus* pertinent to the energetic approach are discussed, including a detailed caloric investigation. Information about the seasonal variation in lipid composition during larval growth was used to explain much of the observed caloric variation. Energy budgets for three of the four instars of *Hedriodiscus* were determined from estimates of growth, respiration, and rates of food ingestion. This information together with population census data permitted calculation of population energy budgets. Efficiencies of energy transfer in both spring communities are compared and discrepancies in the allocation of assimilated energy between populations are attributed to variation in diel behavior patterns and differences in food ingested. Export of *Hedriodiscus* larvae and spider predation on newly emerged adults inflicted heavy mortality and were considered the most important sources of population regulation. The annual energy balance for the two springs was tabulated from estimates of energy income and export. The springs fixed far more energy than was expended in any given year. Greatest losses are attributed to algal export, respiration, and travertine deposition; tree leaves represented a significant income of energy to the springs. Primary production accounted for better than 90% of total income and only 0.5–1.0% of algal primary production was channeled to the dominant herbivore, *Hedriodiscus*. Of algal gross production 20–25% was exported from the system. Photosynthetic efficiency was 1.06% utilization of visible light available to algae.

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LITERATURE pertaining to the ecology of hot spring fauna is relatively scarce, especially when compared with what is known about the ecology of fauna in other aquatic environments. Brues (1924, 1928, 1932) was the first to critically examine the fauna of hot springs in western North America. Setchell (1903) published observations of fauna from hot springs in California and Yellowstone National Park (Y.N.P.) and Lutz (1931) thoroughly examined the fauna of springs near Old Faithful, Y.N.P. Murphy (MS 1957) studied the pattern of community succession on the travertine Mammouth Hot Spring Terraces, Y.N.P.

Investigations of European and North African hot spring fauna are similarly scarce and devoid of ecological interpretation. Issel (1906, 1908) investigated fauna from hot springs in Northern Italy and was one of the few early investigators to mention the existence of a hot springs' community, "fauna termale." Mason (1939) analyzed the distribution of organisms in a permanent thermal gradient in hot springs at Hammam Meskoutine, Algeria. Tuxen (1944) was the first to thoroughly examine the fauna of Iceland's hot springs.

The Ohanapecosh Hot Springs were in a sense a model ecosystem, ideally suited for field study. The paucity of herbivores provided an excellent field situation to test some basic assumptions regarding the flow and efficiency of transfer of energy at both the species and population level (Slobodkin 1960, 1962; Teal 1957; Odum 1957; and Kozlovsky 1968). The simple community

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structure allowed estimates to be obtained of the import and export of energy on an annual basis, and therefore the construction of a community energy budget.

The thermal springs were relatively stable with repeating patterns, and information from several sources indicated little biological or chemical change over the past 100–150 years.

Thus, the overall objectives of the study were to gather information on the ecology of two hot spring communities, noting their similarity and differences and the effects of temperature and food on each.

Chemistry, Geology, and Description of the Study Area

The Ohanapecosh Hot Springs lie in a valley in the southeast corner of Mount Rainier National Park, Washington State, USA (46°44′ N, 121°34′W).

The springs lie near the center of a thick westward dipping section of Ohanapecosh rock, comprised of volcanic clastic rocks and lava flows (Fiske et al. 1963). The Ohanapecosh formation was deposited during the Tertiary Period to an approximate maximum depth of 4520 m in the Mount Rainier area. The geology of Mount Rainier has been well studied by Coombs (1936), Crandell and Waldron (1956), Crandell et al. (1958), and Fiske et al. (1963).

The nine Ohanapecosh Hot Springs are alkaline calcareous springs, deriving most of their water from ground water that leaches various cations from the parent crystallized igneous rock as it ascends the fissures. This dissolution results in the formation of bicarbonates, especially with sodium, potassium, and calcium. The Ohanapecosh Springs deposit travertine ($CaCO_3$), and banks of travertine attain a depth of 7 m in certain areas, testifying to the age of the thermal activity in this region. The temperature of the springs ranges from 35 to 48 C but there is little variation in their water chemistry. A partial chemical analysis of the water from the main study springs for this investigation, springs 4 and 6 (see Fig. 1), together with a cation–anion balance sheet, appear in Table 1.

Springs 4 and 6 were chosen for intensive study because they represented the extremes of a range of community complexity, inversely related to temperature. Spring 4 (47 C) was the least structured biological community whereas spring 6 was the most diverse encountered in the general study area. Common to both springs was the dipteran herbivore *Hedriodiscus truquii* (Bellardi). At the outset it was decided to critically examine *Hedriodiscus* populations in these springs in an attempt to elucidate the role that temperature and food differences may play in affecting their life history and ecology.

During the winter of 1964, a barbed-wire fence was constructed around spring 6 to minimize elk disturbance. Oddly enough, elk were never again seen in the area. Spring 4 was left in its natural state. The flora of spring 4 was predominantly the Cyanophyta species *Schizothrix calcicola* Gom. and *Phormidium* sp.[2] Spring 6 was dominated by *Oedogonium* sp. and associated epiphytic diatoms. The characteristics of the algal community of springs 6 and 3 (similar to spring 4) have been previously described in detail (Stockner 1968a, b). A detailed list of the fauna collected during the study period from nine hot springs at Ohanapecosh, together with taxonomic considerations for the more important species, appear in Table 2. Food web diagrams illustrating the differences in diversity between springs are presented in Fig. 2.

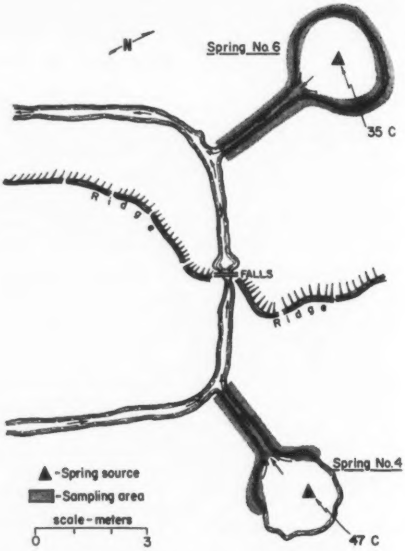

FIG. 1. Study area, springs 4 and 6, Ohanapecosh Hot Springs, Mount Rainier National Park, Washington State, USA.

Materials and Methods

From November to April monthly sampling trips were made; during the summer, biweekly trips were taken, often lasting 5 or more days. Since the animal populations of the springs were nocturnal, a large portion of the observation and collection of fauna was done at night. In 1966 the entire summer was spent at the study site.

Primary production

The diurnal oxygen variation was measured to estimate primary productivity in the shallow runoff streams draining the hot springs. Methods used together with equations and detailed results have previously been discussed (Stockner, 1968b).

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[2] Identification by Dr F. Drouet, Academy of Natural Sciences, Philadelphia, Pennsylvania.

Population census

Changes in numbers of the dominant herbivore *Hedriodiscus truquii* were estimated by haphazardly tossing a 100-cm square quadrat onto the algal mat in the evening and counting the number of larvae enclosed. This process was repeated 10 times and a mean, variance, standard deviation, and confidence limits calculated. Lengths were obtained by measuring larvae and immediately returning them to the algal mat. From regression equations describing the relation of length to wet weight the approximate wet weight estimate was obtained (Fig. 3). Data for calculating these equations were obtained from collections made in 1964 and included well over 500 individual weight measurements. Instar stages were determined by removing the head and measuring its length. Based on a plot of body length to head length instars were separated (Fig. 4). Length-frequency histograms were plotted to characterize shifts in population age structure.

TABLE 1. Summary of partial ion analysis in Ohanapecosh Hot Springs, September 1965.

Constituents	Spring 6	Spring 4
*p*H (laboratory, 25 C)	7.9	7.0
Bicarbonate alkalinity (*mg/liter* $CaCO_3$)	838	852
Carbonate alkalinity (*mg/liter* $CaCO_3$)	0	0
Conductivity (25 C) (*μmhos/cm*)	4570	4590
Iron (*mg/liter* Fe^{++})	0.46	0.06
Sodium (*mg/liter* Na^+)	930	960
Potassium (*mg/liter* K^+)	64	66
Calcium (*mg/liter* Ca^{++})	156	160
Magnesium (*mg/liter* Mg^{++})	3.8	3.8
Sulfate (*mg/liter* $SO_4^=$)	148	176
Chloride (*mg/liter* Cl^-)	870	870

Cation–Anion balance (*values in milliequivalents/liter*)

Ion	Spring 6	Spring 4
Cations:		
H^+	0.0	0.0
Fe^{++}	0.0	0.0
Na^+	40.5	41.7
K^+	1.3	1.3
Ca^{++}	7.3	8.0
Mg^{++}	0.3	0.3
Sum:	49.4	51.3
Anions		
HCO_3^-	16.8	17.0
$CO_3^=$	0.0	0.0
$SO_4^=$	3.1	3.7
Cl^-	24.5	24.5
Sum:	44.4[a]	45.2[a]

[a] Differences between anion–cation are partially attributable to the absence of nitrate determinations.

TABLE 2. Classification of the Arthropoda in Ohanapecosh Hot Springs.

Insecta
  Diptera
    Stratiomyidae
      Hedriodiscus truquii (Bellardi)
      Caloparyphus sp.
    Tipulidae
      Limonia sp.
    Chironomidae
      Chironomus sp.
  Odonata
    Coenagrionidae (= Agrionidae)
      Plathemis lydia (Drury)
  Coleoptera
    Hydrophilidae
      Enochrus carinatus (Horn)
      Laccobius sp.
    Dytiscidae
      Agabus lugens (LeConte)

Crustacea
  Ostracoda
    Cypridae
      Cyprinotus ohanapecoshensis (Ferguson)
  Copepoda
    Cyclopidae
      Paracyclops fimbriatus (Fischer)

Arachnida
  Araneida
    Lycosidae
      Pirata piraticus (Clerck)
    Tetragnathidae
      Tetragnatha versicolor (Walckenaer)

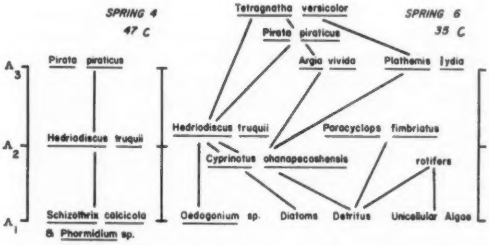
SPRING 4
47 C
Tetragnatha versicolor
SPRING 6
35 C
Pirata piraticus
Λ_3
Pirata piraticus
Argia vivida
Plathemis lydia
Λ_2
Hedriodiscus truquii
Hedriodiscus truquii
Paracyclops fimbriatus
Cyprinotus ohanapecoshensis
rotifers
Λ_1
Schizothrix calcicola
& Phormidium sp.
Oedogonium sp.
Diatoms
Detritus
Unicellular Algae

FIG. 2. Food webs illustrating differences in community structure between springs 4 and 6. The symbol Λ refers to trophic level.

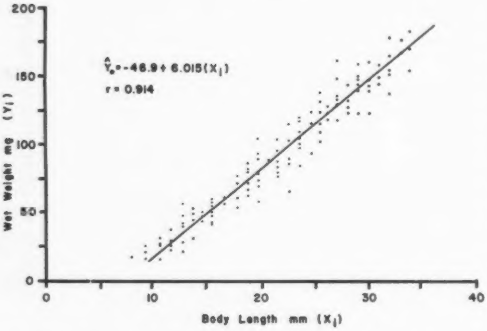

FIG. 3. Regression of *Hedriodiscus truquii* body wet weight (Y) to body length (X).

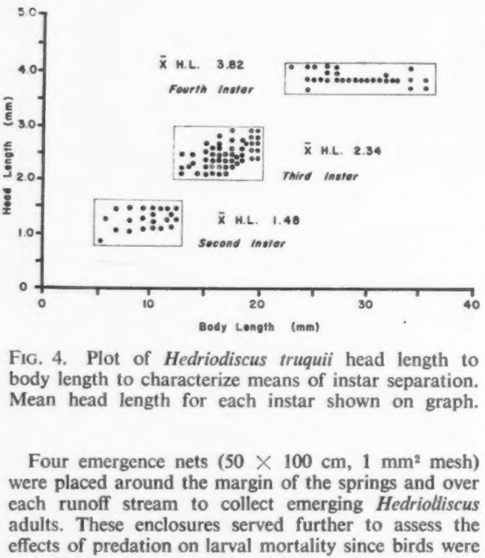

FIG. 4. Plot of *Hedriodiscus truquii* head length to body length to characterize means of instar separation. Mean head length for each instar shown on graph.

Four emergence nets (50 × 100 cm, 1 mm² mesh) were placed around the margin of the springs and over each runoff stream to collect emerging *Hedriodiscus* adults. These enclosures served further to assess the effects of predation on larval mortality since birds were excluded from the area enclosed. Because larvae enclosed by the net could not emigrate, weekly censusing during summer provided a rough estimate of the number of pupae as well as emerging adults.

Feeding rates

Larvae were collected from springs in the evening and placed in numbered petri dishes containing a cushion of moist paper towelling. These dishes were then placed at the spring's margin, covered with black polyethylene and partially submersed in water to keep them near the temperature of the algal mat. After approximately 12 hr the dishes were removed and the weight of larvae measured on a Cahn portable Electrobalance, Model G, with an accuracy of 0.05% of full scale on each range. These larvae were then placed in another petri dish containing fresh algal material taken from the same spring from which the larvae were collected. The dishes were again placed at the margin of the spring and covered with a sheet of black polyethylene. At predetermined intervals, larvae were removed and weighed and then returned to the containers. This procedure was repeated every 2 hr and repeated with fresh algae throughout the duration of the experimental period (3–4 days, twice a month during the summer) Controls consisted of containers with moist paper towelling instead of algal mat. If feces were observed (easily recognized) in the petri dish containing algae, larvae were not weighed but returned to the spring, and the experiment was started again with fresh algae and larvae. The increase in larval weight per unit time provided an estimate of the amount (milligrams) of algal material ingested.

Respiration rates

Respiration was measured in both the field and the laboratory. In the field, organisms were collected, rinsed with spring water to free material clinging to their integument, then placed on an absorbent paper towel to remove excess moisture, and weighed with the Cahn Electrobalance. Larvae were then placed in a cellulose nitrate tube with a capped volume of 53 ml and an O_2 electrode[3] was inserted (Fig. 5). This apparatus was then placed in the spring, which served as a constant temperature bath during the experiment. When respiration was measured in the laboratory, warm tap water

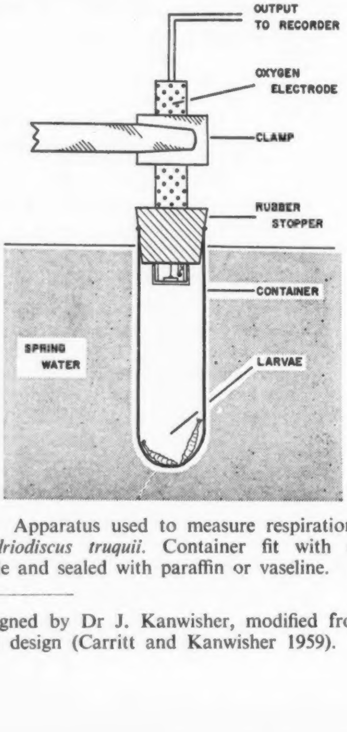

FIG. 5. Apparatus used to measure respiration rates of *Hedriodiscus truquii*. Container fit with oxygen electrode and sealed with paraffin or vaseline.

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[3] Designed by Dr J. Kanwisher, modified from the original design (Carritt and Kanwisher 1959).

was circulated in a beaker containing the apparatus. Since the larvae utilized atmospheric oxygen, stirring within the chamber was not necessary. The duration of experiments depended upon number and weight of larvae in the chamber, ranging from 20 min to 1½ hr.

The oxygen electrode was calibrated to zero in the laboratory with pure nitrogen, and in the field Na_2SO_3 solution. Full scale, 100% saturation, was obtained by allowing the electrode to equilibrate for approximately 1 hr in the atmosphere. A new membrane, either teflon or polyethylene, was put on after 24 hr of continuous use, and the electrode recalibrated. It was then inserted into the tube so as to insure air pressure equalization within the chamber. After insertion, the edges of the tube were sealed with vaseline or paraffin to prevent significant leakage of atmospheric oxygen during the experiments. Output from the O_2 electrode was recorded by a Heathbuilt servo-recorder, Model No. EUW-20A. All data were corrected to standard conditions of temperature and pressure and converted to mm^3/insect weight (mg)·hr, the most common term for expressing insect respiration (Keister and Buck 1964).

Calorimetry

Samples of both flora and fauna were brought back to the laboratory where they were dried in an oven at 60 C, weighed, ground to a fine powder, and burned in a Parr Semimicro Oxygen Calorimeter, Model No. 1411, to determine calories/gram of dry weight and ash-free dry weight. These values were corrected for endothermy due to the considerable ash content (primarily $CaCO_3$) per sample using correction factors discussed by Paine (1966). Seasonal variation in ash and caloric content were determined in 1963, 1964, and the spring of 1965. Dry samples were ashed at 500 C in a muffle furnace for 3 hr.

Lipid analysis

The lipid content of *Hedriodiscus* larvae was determined according to methods of Folch et al. (1956). Slight modifications of the methods were employed to facilitate the rapid treatment of frozen larvae. After filtration the chloroform-methanol solution containing extracted lipid was washed with 0.05 N KCl solution (Raymont et al. 1964), instead of the salt solution called for by Folch. After washing, the solvent was evaporated over a stream of nitrogen.

Organic income and export

Throughout the 1966 sampling program a net (mesh size 1 mm^2) was placed in the main thermal stream draining the major study springs. At hourly intervals the net was removed and collected material was placed in vials and preserved in 10% formalin. At the laboratory this material was sorted under a dissecting microscope into faunal and floral components. The algae were dried and weighed, and then ashed at 500 C, and animals were identified and then treated in a similar fashion.

Quadrats of 1 sq m were placed in each spring and around the perimeter, and allochthonous material (such as leaves, pine needles and cones, and wind-blown vegetation) was collected, placed in plastic bags, and brought back to the laboratory. The material was dried at 40 C, weighed on a beam balance to an accuracy of approximately ±1 g, and aliquots ashed and burned in a bomb calorimeter.

Statistical treatment

Samples collected during the study period were treated with appropriate test statistics after the mean and variance were calculated. In most instances mean values with associated confidence limits were used in the compilation of figures and tables. It was assumed that with an independent mean and variance, these data of continuous variables were normally distributed and thus the *t*-test, regression analyses, and analysis of covariance (ANCOVA) (Snedecor 1956) could be used as appropriate test statistics, without transformation, for the analysis of these data.

Natural History of *Hedriodiscus truquii* (Bellardi) (Stratiomyidae)

Very little is known about the biology of *Hedriodiscus truquii*, and what is known concerns only the adult stages. The larval stage of *Hedriodiscus* has never been described (M. T. James, personal communication).

Eggs

Eggs are laid in early summer by females that have overwintered as third instar larvae and in August by a second generation. They are laid in a cluster at the edge of the springs, generally on top of the algal mat just out of direct contact with flowing water, but moist enough to prevent desiccation. Examination of six clusters showed the mean number of eggs per cluster to be 165 (SE 25). The eggs are pale yellow and approximately 1 mm long and adhere easily to any object via a mucilaginous coating. The hatching time for the eggs was between 4 and 9 days.

Larvae

Irwin-Smith (1920) noted that upon emergence from the egg, newly hatched larvae molted immediately to the second instar. I was unable to confirm this observation for *Hedriodiscus*. The smallest larvae collected during the study were 6 mm long and weighed only 7 mg (wet weight). The maximum length attained by larvae in field collections was 38 mm and maximum weight was 189 mg. A regression of length to weight revealed a significant correlation of 0.914 (Fig. 3).

There are four instars but only three could be determined from field collections. These were determined by measuring the hard sclerotized head capsules. A plot of head length to body length further characterizes the instars (Fig. 4). The ratio from one instar to the succeeding one was approximately 1.60.

The larvae are herbivorous, feeding almost exclusively on algae. Food is swept into the oral cavity by the mandibular-maxillary complex, which is located in the pocket formed by the labrum and the extension of the optic lobes (Fig. 6A). This appendage also serves as the larva's only means of locomotion. Their rate of movement is slow when feeding during the night.

Larvae are helpless in swift water and when swept off the mat by a surge of current they float on the surface, held there by a rosette of fine hairs surrounding the siphon spiracles (Fig. 6B(*a*)). This "export" of larvae from the algal mats represents a significant mortality factor that will be discussed in a later section. Since larvae are unable to cope with swift current and each of the Ohanapecosh springs are connected, *Hedriodiscus* larval populations in each spring are isolated from each other. Because the larvae avoid swift current and deep stagnant water, they are always distributed either on algal mats in the small runoff streams or near the periphery of spring pools in very shallow water. Larvae feed during the night and by day are hidden either beneath the mat or under allochthonous material along the spring margin.

Pupae

The pupa is coarctate, and the stage is passed within the last larval skin (fourth instar), termed a puparium (Fig. 6C). At the onset of pupation mature fourth instar larvae become rigid and immobile. The exact time from the onset of pupation to adult emergence is unknown but probably ranges from 7 to 11 days. Swammerdam (1737) noted that 11 days were required for *Stratiomys chamaeleon* to complete the cycle, and that about 25 min were required for the adult fly to break through and crawl out of the puparium, and an additional hour before flight could occur.

During the pupation *Hedriodiscus* is directly exposed to predators. The period of adult emergence is a crucial one, for the carnivorous spiders *Pirata piraticus* and *Tetragnatha versicolor*, common on the periphery of the mats and in grasses surrounding the springs, are attracted to the movements of emerging adults. Scarcity of adults in emergence nets and around the springs is due chiefly to this factor.

Adults

The adult female is larger than the male and can be readily identified and separated on the basis of abdominal coloration (Fig. 6D). Adults are good fliers, often hovering near vegetation surrounding the springs. Evidence indicates that the adult life consists of little beyond mating and laying eggs (Oldroyd 1964).

The duration of adult existence is unknown but is probably on the order of a few days (M. T. James, personal communication). From larval census data presented and discussed in later sections it appears that adults copulate soon after emergence, and that within a day or two the female lays her eggs on the algal mat.

Predators and parasites

Birds often forage on the algal mats but their main prey are either odonates or the small dipteran *Caloparyphus* sp. (Stratiomyidae). Spiders are common around the springs and take their share of emerging adult insects, including *Hedriodiscus*. *Hedriodiscus* larvae are not preyed upon by spiders. This is likely due to their extremely tough calcium carbonate-impregnated integument and their nocturnal activity period. The soft pupae and emerging adults, however, are highly vulnerable and easily taken.

Diapause

Hedriodiscus larvae commence diapause around the middle of October and do not become active again until the end of March or early April. The diapause period is spent either around the margin of the spring under allochthonous material or buried in the soft sediment in the spring pools.

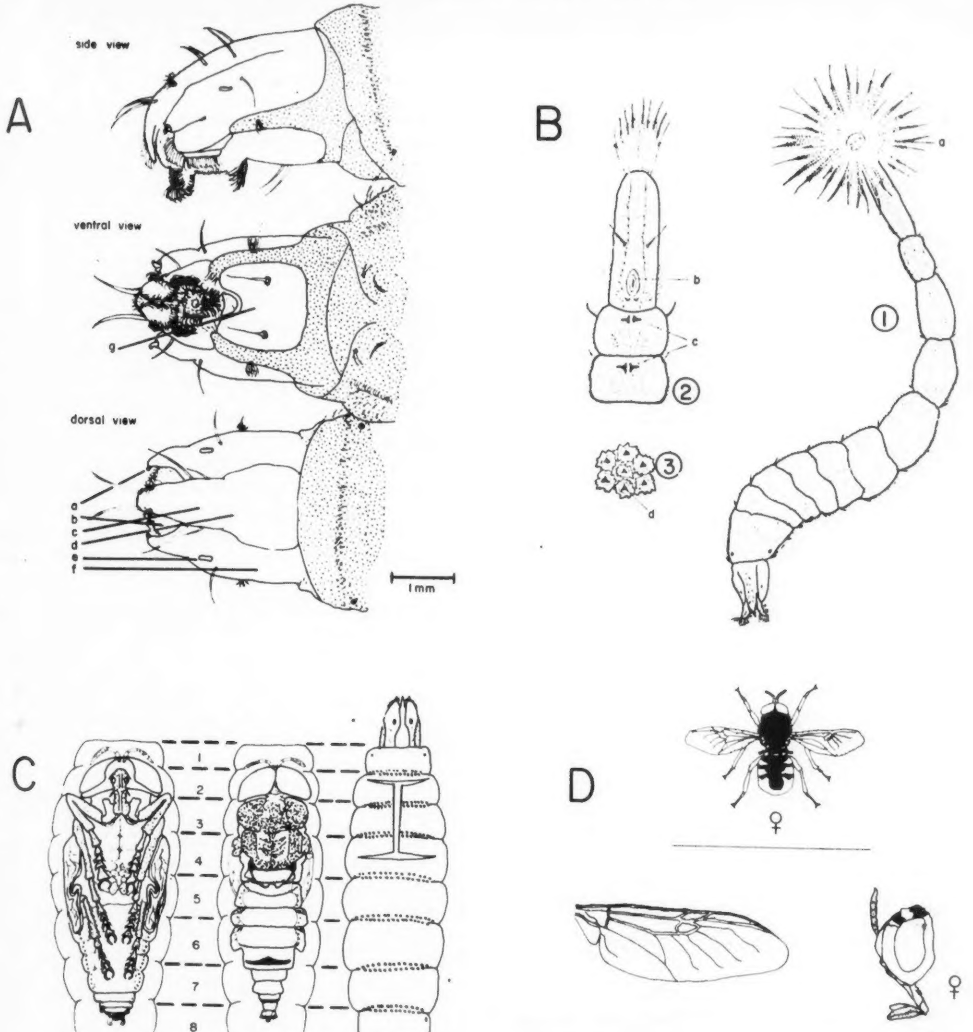
Caloric studies

There is a direct relation between the caloric content and wet weight of larvae (Fig. 7). This relation suggests that a significant portion of their caloric income is converted to lipid for storage. There is a distinct seasonal change in caloric content of larval *Hedriodiscus* populations (Fig. 8) that is the result of a gradual accumulation of lipid in third and fourth instars.

Caloric values determined for algal material, imported allochthonous material, *Hedriodiscus* eggs, larvae, pupae, and adults, and other invertebrates common to most of the springs are shown in Table 3.

Lipid analysis

As previously discussed, fat accumulation is a characteristic feature of the internal anatomy of *Hedriodiscus* and accounts for the increased caloric content in latter instars. There is a progressive


A
side view
ventral view
g
dorsal view
a
b
c
d
e
f
1mm
B
a
b
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②
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4
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8
9
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FIG. 6. (A) Head of *Hedriodiscus truquii* larva in side, ventral, and dorsal view: (*a*) antenna; (*b*) mandibular–maxillary complex; (*c*) labrum; (*d*) clypeus; (*e*) eye; (*f*) ocular lobe; (*g*) submentum. (B) *H. truquii* larva: (1) entire larva; (2) ventral view of abdominal segments 6 and 7, and apical segment; (3) enlargement of integument; (*a*) spiracular chamber; (*b*) anal opening; (*c*) sclerotized hooks; (*d*) calcareous nails (1 and 3 after Swammerdam 1737; 2, original). (C) Pupa of *H. truquii* within fourth instar cast (puparium): (1) ventral view; (2) dorsal view; (3) fourth instar cast with H-shaped opening, dorsal view. (D) Adult, female *H. truquii*, including enlargements of head appendages, wing venation, and male and female body patterns.

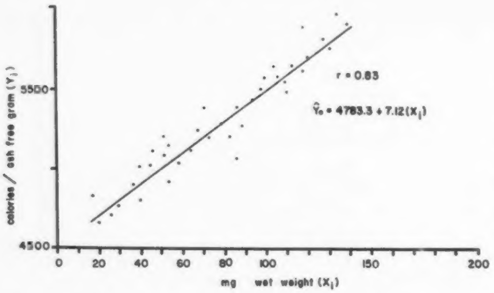


FIG. 7. Regression of *Hedriodiscus truquii* larvae wet weight (X) to calories/ash free gram caloric content (Y).

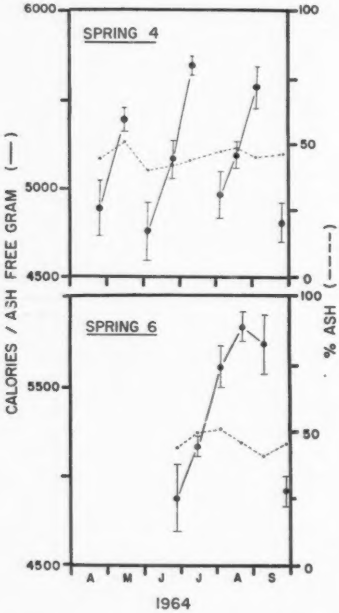


FIG. 8. Seasonal caloric variation and ash content of *Hedriodiscus truquii* populations in springs 4 and 6 in 1964.

increase in percentage lipid composition from second to fourth instars (Fig. 9). Approximately 20% of a fourth instar's dry body weight is composed of lipid and between 5 and 10% of a second instar larvae. Mean values for adults and pupae plus cast are 13 and 16% respectively. In terms of total organic body content (dry weight), lipid comprises almost 35% of the organic matter in fourth instar larvae but less than 15% in second instar larvae.

Population Ecology and Energetics of *Hedriodiscus truquii*

ECOLOGY OF HEDRIODISCUS TRUQUII

Populations of *Hedriodiscus* inhabiting springs 4 and 6 were compared to ascertain what effect temperature and different algal food material would have on respiration, numbers, growth rate, and caloric content.

Population density

Characteristically there are two generations per year with adult emergence occurring near the end of June and again in the last 2 weeks of August. This basic pattern from field data collected in 1965 can be seen in Fig. 11. In 1964 major departures from this pattern were observed (Fig. 10). In the winter of 1963–64 elk trampled the stream channel and pool of spring 6, inflicting a heavy mortality on overwintering larval populations. No *Hedriodiscus* larvae were observed until June 28 of that year. This generation was probably started from eggs deposited in spring 6 by females emerging from adjacent springs.

In spring 4 during the winter of 1963–64 a young elk fell through the weak timbers covering the source and was parboiled. Heavy snows precluded removal until April 1964. The algal mat receiving the liberated decomposition products was especially thick, and the caloric content of the mat was significantly higher than recorded in 1965 or 1966 (Table 3). *Hedriodiscus* larvae, feeding on this enriched mat went through three generations instead of the usual two observed in 1965 and 1966 (Fig. 11). This fortuitous circumstance provided reasonable evidence of the limitation of population growth by food quality. The importance of this factor will be discussed in later sections. A regression of larval length to weight was calculated for both springs from data obtained in 1964 (Fig. 12). The slopes of these regressions were compared and were found to be significantly different (ANCOVA, $P = 0.05$). This suggests that per unit length, larvae in spring 4 were significantly heavier than those in spring 6.

Since specimens had to be removed from springs at each sampling interval during 1964, it was thought that an accurate picture of population density fluctuations could not be obtained, as in effect the sampler was acting as chief predator. But despite the removal of some larvae from the springs, there was no discernable difference in density between years 1964–65. In 1965 and 1966 after larvae were measured they were returned to the mat. Peak densities in spring 4 occurred at two periods, May 7, 1965 ($12/m^2$), and July 1, 1965

TABLE 3. Calorific values of components of the communities in two springs of Ohanapecosh Hot Springs.

Material	No. of determinations	% ash	calories/g	calories/ ash free g	SE calories/g
		Hedriodiscus truquii			
1st instar (by extrapolation)	—	—	2258	4608	—
2nd instar	16	48.0	2470	4750	97
3rd instar	21	46.5	2726	5096	63
4th instar	29	42.0	2669	5560	73
Adults (male and female)	13	10.0	4927	5375	102
Cast (exoskeleton material; all stages)	8	76.0	764	3185	91
Eggs	2	2.0	5845	5964	231
Feces	2	85.0	681	4544	113
		Invertebrates			
Enochrus carinatus	7	9.1	5371	5908	54
Argia vivida	13	14.5	5075	5936	61
Plathemis lydia	6	13.0	5098	5860	101
Pirata piraticus	4	7.2	4886	5265	96
Tetragnatha versicolor	4	8.0	5164	5613	136
Caloparyphus sp.	18	34.0	3862	5852	74
		Flora			
Maple leaves	3	9.7	4773	5290	102
Export material	6	76.0	1034	4312	88
Schizothrix calcicola (spring 3)	31	72.4	1264	4543	61
Oedogonium sp. plus diatoms (spring 6)	8	72.3	1134	4036	101
Oscillatoria terebriformis	7	71.9	1249	4405	96
Schizothrix calcicola and *Phormidium* sp.					
(spring 4, 1964)	8	68.0	1588	4963	132
(spring 4, 1965–66)	5	70.0	1356	4761	154

(26/m²) (Fig. 13). In 1966 the greatest density was observed on May 2 (17/m²) and July 3 (23/m²). The population of each generation declined to a level of less than 7/m² on June 17, 1965, and 13/m² on August 28, 1965, and in 1966 to 7/m² on June 18 and 7/m² on September 6. The repetition of the general bimodal nature of these density curves for both 1965 and 1966 is further evidence of the characteristic pattern of two generations per year.

Adult densities obtained from emergence traps during the emergence periods were extremely low for both springs: 1.2/m² and 2.8/m² on July 1 and August 26, 1965, respectively. In 1966 adult densities were similar to those observed in 1965. The density of larvae per unit area in spring 6 was higher than in spring 4, but adult densities were nearly identical in both springs. Attempts to census the adult population by using a sweep net in grasses adjacent to the springs proved unsuccessful. In 2 years only one adult was obtained by this method.

Population age structure

The larval populations at any given sampling consisted of not more than two instars represented in varying proportions. This is likely due to both variability in hatching period and time required for second instar larvae to reach the algal mat and commence feeding. A comparison of the frequency of instars sampled from each spring during 1964,

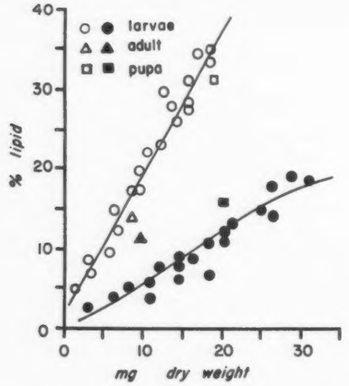
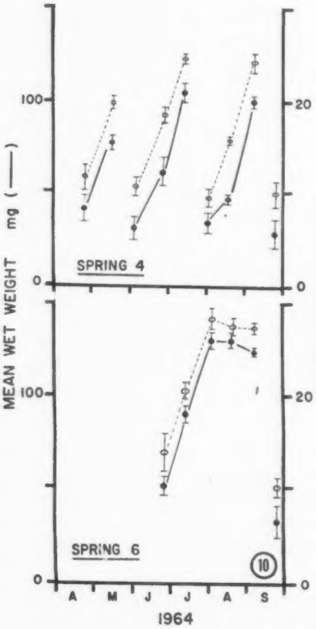


FIG. 9. Percent lipid content of *Hedriodiscus truquii* larvae, pupa, and adult. Solid symbols represent lipid content on mg dry weight basis, and open symbols on a mg organic matter, dry weight basis.

1965, and 1966 is presented in Fig. 14. The clear nonoverlapping progression of larval instars indicates that from initial egg deposition to adult emergence, no further inoculum of eggs is received on the mat. If this were so then no readily observable pattern would be apparent.

Mortality

From the first observation of larvae to adult emergence, decreases in larval populations are equivalent to mortality (export is included in this category). Rates of mortality are given by slopes of regression lines fitted to these data for 1965 and 1966 (Fig. 15). Calculated mortality rates were comparable for the two springs, but tended to be higher for the second generation in both years. This is chiefly due to increases in larval export in August and early September, and concomitant increases in the major predator *Pirata*. Export data obtained concurrently indicates that up to 90% of the ob-



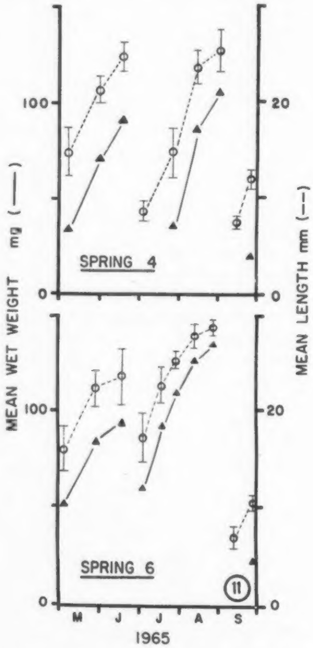


FIG. 10. Seasonal changes in mean *Hedriodiscus truquii* larvae wet weight and mean body length in springs 4 and 6 in 1964. Note occurrence of only one generation in spring 6 and three generations in spring 4.

FIG. 11. Seasonal changes in mean *Hedriodiscus truquii* larvae wet weight and mean body length in springs 4 and 6 in 1965. ▲ is body weight estimated from regression equation shown in Fig. 3. Note the two generations/year in both springs.

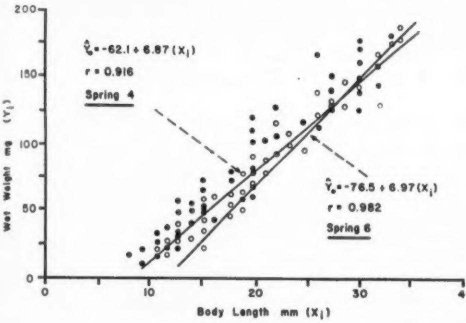


FIG. 12. Regression of *Hedriodiscus truquii* body weight (Y) to body length (X) in springs 4 and 6 in 1964. Slopes compared (ANCOVA) and dissimilar at $P = 0.05$ level.

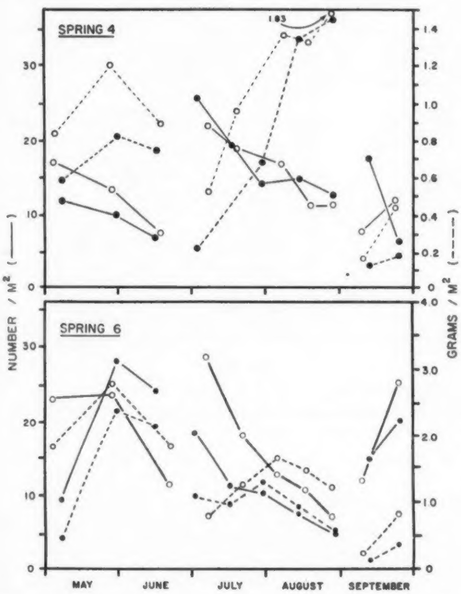


FIG. 13. Seasonal changes in numbers and biomass/m² of *Hedriodiscus truquii* larvae in springs 4 and 6 in 1965 (●) and 1966 (○).

served mortality is directly attributable to this factor.

Periods of pupation and adult emergence are crucial and mortality rates attest to the validity of this assertion (Fig. 16). Slopes of regression lines represent instantaneous rates for the brief period of fourth instar pupation and adult emergence. If all larvae pupated at the same time and adults emerged synchronously on the same day, only to be preyed upon by spiders immediately upon emergence, then these rates would not accurately depict mortality occurring during this period. However, this synchrony is not prevalent, and from field evidence it appears that pupation and emergence occurs randomly throughout the 2-week pupation period. Extrapolation of cage mortality rates to the spring community as a whole assumes that all possible sources of mortality were represented within the cage, and that the area enclosed was representative of any given sampling area. Such predators as birds were of course unavoidably excluded, but the spiders *Pirata* and *Tetragnatha* were both included under the net. Quantitative estimates of the extent of bird predation could not be obtained.

Immigration and flushing

Since all *Hedriodiscus* natality took place at two time periods, the last 2 weeks of June and August, the observed decrease in population density must be due to mortality, including emigration. *Hedriodiscus* lacks a suitable means of locomotion to cope with the strong current separating each spring and larvae seldom leave the moist spring margins. Thus, immigration from adjacent springs is a negligible factor affecting population density fluctuations. However, export, or loss of larvae swept from the mat by currents is of considerable importance and as has been discussed, represents the most important mortality source.

Production

Ideally production consists of all organic products synthesized and stored by an organism or population. Inherent difficulties involved in separating these products from egested material requires a restriction of the definition to include only (1) growth, or increase in larval biomass per unit time, and (2) exoskeleton or cast material discarded during each molt.

1. *Growth*—Regression lines fit to field data showed an exponential growth rate in both springs during 1964, 1965, and 1966 (Fig. 17). The maximum growth, 2.12 mg/insect·day, occurred in spring 4 in 1964 from larvae feeding on the enriched algal mat. The lowest rates were obtained in both springs in all years during the fall. The annual mean growth rate for spring 4 was 1.67 mg/insect·day and for spring 6 1.27 mg/insect·day. This difference was statistically significant (*t*-test, $P = 0.05$ level). Differences in growth observed between springs was chiefly attributable to food quality and temperature, and will be discussed in later sections.

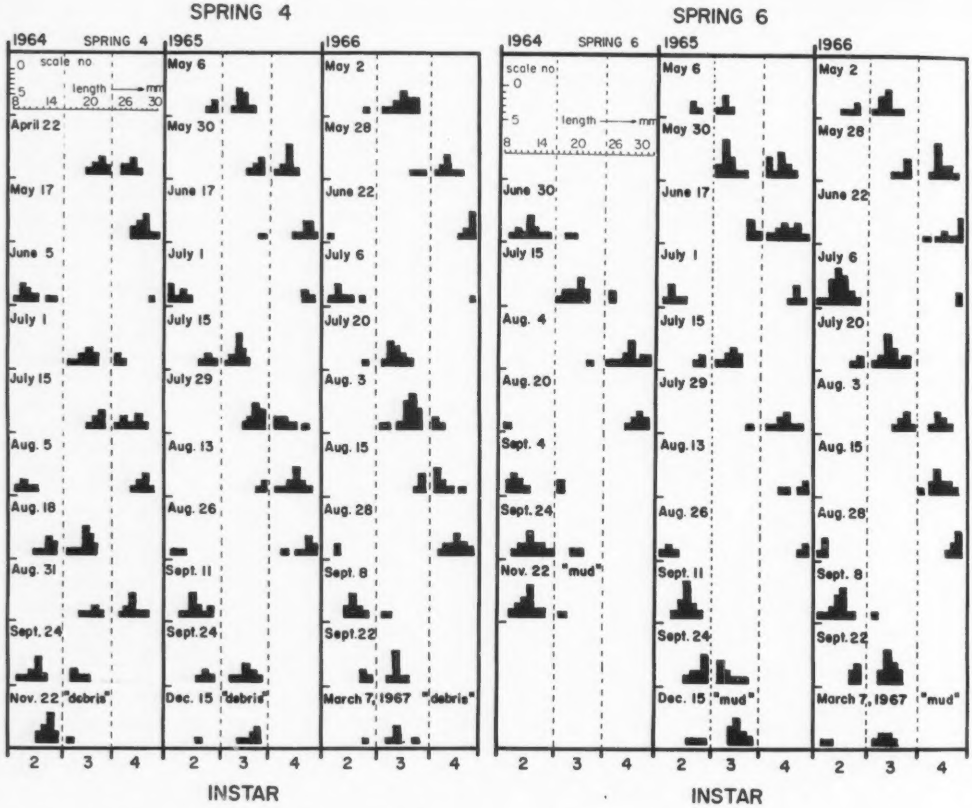


FIG. 14. Histogram of *Hedriodiscus truquii* larval density showing seasonal distribution of instar stages 2, 3, and 4 in springs 4 and 6 in 1964, 1965, and 1966. (Scale in upper left-hand corner of figure.)

2. *Larval casts* — The mean weight was calculated for casts of instars 2, 3, and 4, and estimated by extrapolation for instar 1. A semilog plot of these data, together with mean body wet weight of each instar, essentially showed the same rates of increase (Fig. 18). The weight of the second instar cast was 12.8% of the mean body weight of that instar, and the fourth instar molt (puparium) was 15.2% of the mean body weight. The mean body weight to cast weight ratio for instar stages 2, 3, and 4 was approximately 7:1.

Respiratory rates

A log-log plot of body weight to respiratory rate showed a linear relation up to a weight of 40 mg above which there was no further increase (Fig. 19). The mean rate of O_2 consumption for second and third instar larvae was proportional to body weight raised to the power 0.91; however, the ratio decreases in fourth instar larvae and adults to a power of 0.62, presumably reflecting an increase in metabolically inert lipids and an increase in exoskeleton material in adults. Oxygen consumption by *Hedriodiscus* larvae decreased from a mean of 0.72 mm^3 O_2/mg·hour for second and third instars to 0.27 mm^3 O_2/mg·hour for fourth instars and adults.

It is difficult to determine what constitutes "normal" or "basal metabolism" in an insect, and there is room for considerable variation when measuring insect respiration rates. For example, respiration falls steadily after an extended period of starvation (Clarke 1957), and will fluctuate depending upon the stage and condition of larvae, i.e., resting prior to molt versus newly emerged larvae actively feeding. Much of the variation in data of insect metabolism is discussed by Keister and Buck (1964).

Temperature variation had little effect on the respiration of larvae and adults. Larvae taken from spring 4 at an algal mat temperature of 41 ± 1 C showed little change in respiratory rate over a range of temperatures from 25 to 40 C. At a temperature of 45 C two results were obtained: (1) larvae became either quiescent with no apparent change (no slope in recorded line), or (2) were

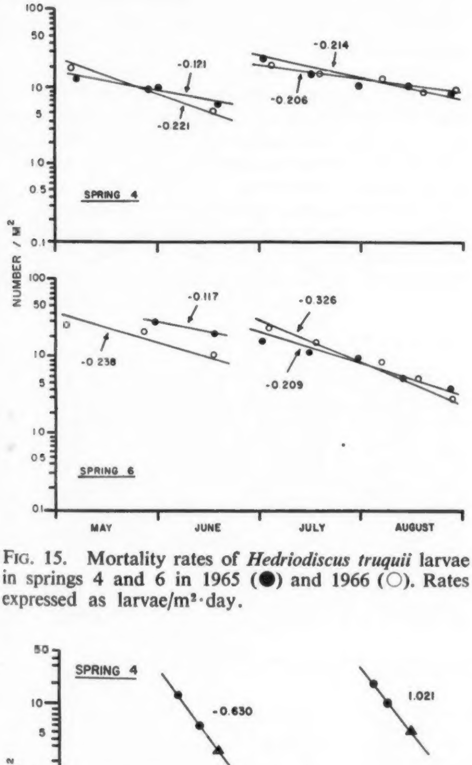


FIG. 15. Mortality rates of *Hedriodiscus truquii* larvae in springs 4 and 6 in 1965 (●) and 1966 (○). Rates expressed as larvae/m²·day.

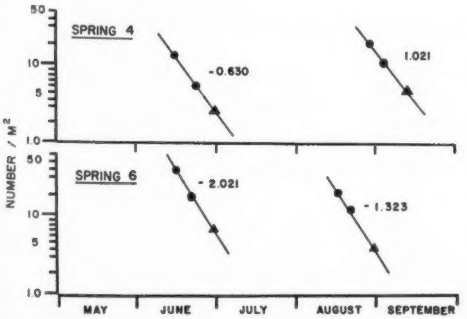


FIG. 16. *Hedriodiscus truquii* mortality rates during the period of fourth instar pupation (●) and adult emergence (▲), 1965. Rates calculated from census within emergence traps, and expressed as insects/m²·day.

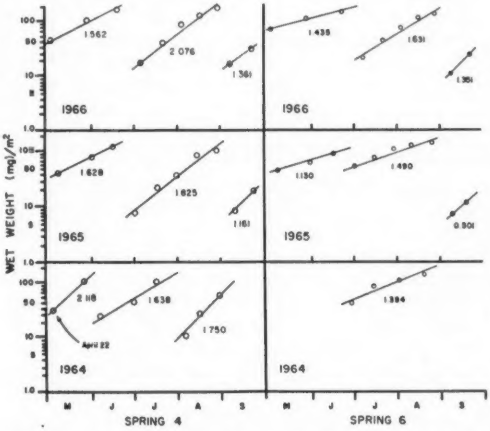


FIG. 17. Growth rates of *Hedriodiscus truquii* calculated from field observation in springs 4 and 6 in 1964, 1965, and 1966. Rates expressed as mg/insect·day.

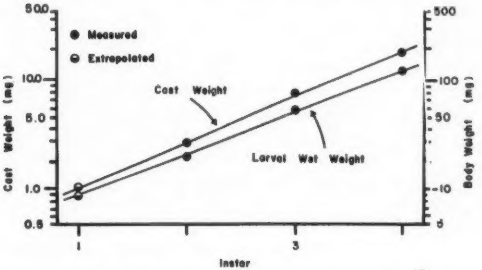


FIG. 18. Semilog plot of *Hedriodiscus truquii* body wet weight and cast wet weight for each instar stage.

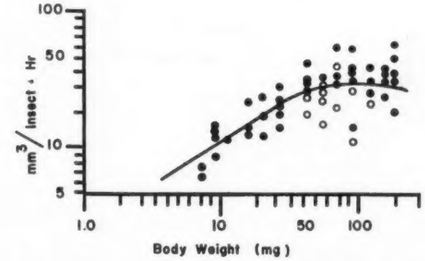


FIG. 19. Respiration rates of *Hedriodiscus truquii* larvae (●) and resting adults (○) as a function of body wet weight. Line fit by eye.

hyperactive, respiring in bursts (steep, steplike slope in line). Part of this response may be due to malfunction of the oxygen electrode at high temperatures, but more likely it represents the high temperature "break point" typical of respiration–temperature curves (Keister and Buck 1964). Larvae taken from spring 6 with a mean temperature of 35 C exhibited a similar break point but at 42 instead of 45 C. Larvae from spring 6 had a Q_{10} of 1.34 that ranged from 1.21 to 1.46 from 30 to 40 C. There was no discernible change in rate between 20 and 30 C ($Q_{10} = 1.0$).

These Q_{10} data suggest that larvae possess a wide range of temperature metabolic adaptation, presumably the result of their past history of temperature exposure. In their life history they are subject to a wide range of temperatures, beginning as first instar larvae out of contact with warm water, exposed to ambient temperature fluctuation. When they commence feeding in the spring they are subject to only slight variation in temperature, but again at the onset of pupation they are subject to temperature fluctuations.

Insects are considered to have little ability to compensate for environmental temperature (Scholander et al. 1953). Keister and Buck (1964) found no difference in the response to temperature changes between *Phormia* sp. kept at 6 C for several days and those taken directly from 25 C. The slight variation in larval respiration rates when taken over three 10-C temperature intervals (10–20, 20–30, 30–40) suggests a similar lack of temperature–metabolic compensation in *Hedriodiscus*. Since the effect on the rate of respiratory energy loss due to the variation of larval activity could not be precisely assessed, extrapolation of these respiration data to field populations is subject to some error.

Rates of ingestion and egestion

Results of feeding experiments with instars 2, 3, and 4 showed a significantly higher (t-test, $P = 0.05$) feeding rate among larvae taken from spring 6 (Table 4). However, when larvae from spring 6 were transferred to dishes containing spring 4 algal mat and placed at higher temperatures the rate was no longer significantly different ($P = 0.01$). This difference in feeding reflects the relative ease with which larvae can graze filamentous *Oedogonium* sp. and epiphytic diatoms as compared with the smooth, fibrous blue-green mat from spring 4.

Despite differences in feeding rates, larvae from both springs take in similar amounts of energy per day. This is due to the higher caloric content per gram of algal mat in spring 4 and also the lower ash content (compare caloric values for algae in both springs, Table 3).

Rates of egestion calculated by weighing fecal pellets discharged per day were not significantly different. These data, obtained concurrently with feeding rate data, are presented in Table 4.

Reproduction

Oviposition by females occurred at two periods: one commencing around the last week in June and the other the last week in August. The number of eggs per female ranged from 140 to 190, with a mean of 165. These estimates are undoubtedly low because it was difficult to establish whether the female had laid eggs prior to dissection or whether any one of the clusters examined represented the total reproductive output of one female.

Hatching success from eggs is poor as shown by information obtained from an emergence trap

TABLE 4. Ingestion and egestion rates of *Hedriodiscus truquii* in two springs of Ohanapecosh Hot Springs.

Stage	No. averaged	Temp (*C*)	Body wt (*mg*)	Mean ingestion/ day (*mg*)	SE ingestion	Mean egestion/ day (*mg*)	SE egestion
			Spring 6				
Second instar	8	34	31.4	8.5	2.5	6.2	3.1
Third instar	14	35	72.6	18.1	3.4	13.8	1.6
Fourth instar	27	35	124.5	24.1	2.0	17.6	1.0
			Spring 4				
Second instar	4	41	30.0	7.0	3.1	5.0	2.8
Third instar	15	40	70.5	14.4	1.7	10.4	2.6
Fourth instar	28	41	121.4	17.8	2.7	13.5	4.1

placed on the margin of spring 6. A cluster of approximately 120 eggs was observed within the net at daily intervals and the first larvae seen were small (7 mm) second instars, at an average density of 21/m^2. Thus, from the original deposition, only 18% survived to commence feeding. This field estimate of hatching success may be high, since within the emergence net the eggs and young larvae were not subject to normal environmental pressures, i.e., sloughing, disturbance, or heavy rains. Field collections, however, validate this estimate of survival since the density of second instars/m^2 at first observance was never greater than 30/m^2.

The sex ratio of adults captured from emergence traps was determined by abdominal coloration. Nets often contained a greater number of males than females, but the ratio did not differ significantly from 50:50 ($\chi^2 = 1.66$, $P = 0.05$, 3 df).

Using 165 as an estimate of mean number of eggs per female the estimated number of eggs deposited per m^2 for the two emergence periods ranged from 140 to 355 eggs/m^2. This estimate assumes that the number of females emerging per m^2 deposit their eggs in their "parent" spring. This fact has not been established.

Caloric values

A respiratory quotient (R.Q.) for *Hedriodiscus* was not calculated. Instead of using an R.Q. of 0.82, typical of protein metabolism, a slightly higher quotient was chosen, R.Q. 0.96, indicative of the conversion of carbohydrate to fat. The conversion of carbohydrate to fat is doubtless the principal metabolic pathway in *Hedriodiscus* larvae and therefore the R.Q. selected does not seem unreasonably high. The oxicaloric coefficient used in this study was 4.9 cal/ml of oxygen consumed, corresponding to the R.Q. of 0.96 (Brody 1945, p. 310). Caloric equivalents of oxygen consumption by second, third, fourth instars, and adults are given in Table 5. These values were used in the calculations of individual and population energy budgets. Mean caloric values of related material important in the calculation of the energy budgets are given in Table 3.

Individual larval energy budgets

Definitions of ingestion, production, egestion, and respiration as set forth by Wiegert (1964) were slightly modified and used in this study: *ingestion* — gross energy intake (caloric content of food); *egestion* — ingestion (respiration plus production); *assimulation* — ingestion-egestion (metabolizable energy); *respiration* — maintenance costs; *production* — energy contained in organic material synthesized by the organism; *growth* — that portion of production incorporated as an increase in the biomass of the organism (excludes molted casts, secretion products, etc.).

Energy budgets for instars 2, 3, and 4 of *Hedriodiscus* populations in springs 4 and 6 were compared and were found to be similar (Table 6). First instar larvae were scarce and budgets could not be accurately determined. The fraction of ingested material assimilated by larval populations in spring 4 was slightly higher than in spring 6, chiefly due to differences in food material between springs. It is assumed that adult *Hedriodiscus* do not feed, and that therefore their budget is simply the daily utilization of high energy products, especially lipid, stored in the larval stage. Based on this

TABLE 5. Oxygen consumption by *Hedriodiscus truquii* and caloric equivalents.

Stage	No. averaged	Mean wt mg (*wet wt*)	ml/g·hr (*mg/g·hr*)	mm^3/insect·hr	calories/day
1st instar[a]		12.0	1.03 (1.47)	12.36	1.41
2nd instar	13	23.4	0.81 (1.16)	18.95	2.30
3rd instar	21	52.6	0.61 (0.37)	32.07	3.83
4th instar	16	118.5	0.21 (0.30)	24.88	2.94
Adult (resting)	9	83.2	0.26 (0.37)	19.14	2.23

[a] All figures in this row are by extrapolation.

TABLE 6. Energy budgets: *Hedriodiscus truquii*. Values in calories/insect per day.

Stage	Ingestion	Respiration (R)	Growth (G)[a]	Egestion (E)	R+G+E	% assimilation
			Spring 6, mean temp 35 C			
2nd instar	6.69	2.11	2.08	2.50	6.69	63
3rd instar	10.52	3.76	3.60	3.16	10.52	70
4th instar	13.46	2.82	4.12	6.52	13.46	53
			Spring 4, mean mat temp 41 C			
2nd instar	9.61	2.49	3.50	3.62	9.61	62
3rd instar	12.74	3.90	4.36	4.48	12.76	65
4th instar	13.81	3.01	5.12	5.68	13.81	59

[a]By difference.

assumption resting adults (nonflight) use 0.7% of their body's total caloric content per day.

The percent assimilation was higher for instars 2 and 3 than for instar 4, averaging about 65%. The average assimilation of 55% for fourth instar larvae is due to a noticeable decrease in activity that is characteristic of these larvae as they near the onset of pupation. The observed variation in instar assimilation is not surprising in light of available information on variation in insect metabolism, the most notable example provided by Wiegert (1964) who obtained a wide range of assimilation rates for *Philanenus* nymphs.

Hedriodiscus population energy budgets

The following equalities were used in calculating *Hedriodiscus* population energy budgets (modified from Wiegert 1964):

(*a*) *Larvae from previous fall*: the caloric value of overwintering larval populations from estimates of mean density per m^2 obtained from winter samples. (*b*) *Growth*: summation of mean numbers per m^2 per sampling interval times the instantaneous growth rate of their respective larval instar stage. (*c*) *Molting losses*: total weight of casts produced per year times caloric value of cast material. (*d*) *Respiration*: summation over all sampling intervals of mean number of larvae per m^2 in each larval instar stage times their respective respiration caloric equivalents. (*e*) *Adult respiration*: summation over all sampling intervals of the mean number of adults per m^2 times their respiration (resting value) caloric equivalent (this represents a minimal value; oxygen consumption during flight would be considerably higher). (*f*) *Production*: growth (*b*) plus molting losses (*c*). (*g*) *Assimilation*: respiration (*d*) plus production (*f*). (*h*) *Ingestion*: assimilation (*g*) times 1.75. (*i*) *Egestion*: ingestion (*h*) minus assimilation (*g*). (*j*) *Increment to adults*: energy content per adult times the number of fourth instars successfully pupating. (*k*) *Mortality* (predator induced): growth (*b*) minus the increment to adults (*j*) times 0.1. (*l*) *Mortality* (export): growth (*b*) minus increment to adults (*j*) times 0.9.

The amount of energy transformed by *Hedriodiscus* populations in spring 4 was a bit higher for both years (Table 7). This is attributable to several factors, the most important being increased respiration, and higher average adult densities. The combined energy flow of *Hedriodiscus* populations in 1966 was greater than in 1965 and this may be partially due to more favorable weather conditions (more sun) in 1966, which would in turn have a greater effect on both primary and secondary production.

Energy flow and efficiencies of energy transfer

The definition of energy flow used in the following discussion is:

energy flow = production + respiration during time (t).

It follows that an estimate of secondary production would be (after Kaczmarek 1967):

production = assimilation − respiration during time (t).

TABLE 7. *Hedriodiscus truquii* population energy budgets, 1965, 1966 (calories/m² year).

	Spring 6		Spring 4	
	1965	1966	1965	1966
Growth	2004	2208	3919	4885
Exoskeleton	87	99	172	214
Respiration	6387	7779	6950	7387
Production	2091	2308	4091	5099
Assimilation	8478	10086	11041	12486
Ingestion	14921	16944	18438	20852
Egestion	6443	6858	7397	8365
Increment to adults	612	847	1039	1211
Larvae from 1964, 1965 (overwintering)	361	241	401	288
Mortality	1479	1361	2592	3329
Export mortality	1332	1224	2333	2996
Natural mortality	147	137	259	333

The definition of energy flow as here considered is that fraction of assimilated energy stored and respired by the population during time *t*. Production is that portion of assimilated energy stored by the population during time *t*, and is not at this time used for respiration.

The numbers, biomass, and energy flow/m²·day for both springs are compared for the years 1965 and 1966 (Fig. 20). Both springs showed peak larval densities in July of both years, attributable to the increased number of small larvae hatching from eggs laid at the end of June. Two periods of increased biomass are evident in both springs during both years, the major one occurring at the end of May in spring 6, and in the middle of August in spring 4.

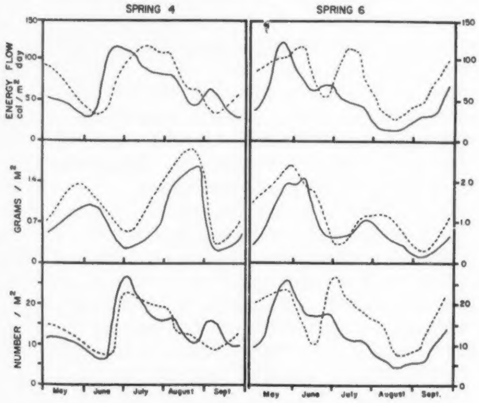


FIG. 20. Seasonal variation in energy flow, numbers, and biomass of *Hedriodiscus truquii* populations in springs 4 and 6 in 1965 (solid line) and 1966 (broken line). Line smoothed and fit by eye.

Energy flow tended to parallel the curve of numbers rather than biomass and this is chiefly due to higher respiration and growth rates in second and third instar larvae. Peak energy flow in spring 4 occurred from the end of June to the middle of August, bracketing the period of maximum population growth. In spring 6, maximum energy flow occurred both from May to the middle of June and from June to July, both periods of increased growth of second and third instar larvae. The periods of adult emergence, June and August, are characterized by substantial decreases in numbers, biomass, and energy flow. Peak periods of energy flow also tended to coincide with periods of maximum algal standing stock and primary production. Teal (1957), Golley (1960), and Wiegert (1964) found a similar correlation in their herbivore energy flow studies.

Efficiencies of population energy transfer

Growth efficiencies were calculated for *Hedriodiscus* populations in both springs from data presented in Table 7. Efficiencies varied between springs, but very little between years (Table 8). The increase in biomass accounted for about 13% of the energy ingested or 23% of the energy assimilated by *Hedriodiscus* populations in spring 6. Populations in spring 4 put more energy of food

TABLE 8. Efficiencies of population energy transfer, 1965 and 1966.

Efficiencies	EE No.[a]	Spring 6		Spring 4	
		1965	1966	1965	1966
Growth/ingestion (NP_n/I_n)	4-D	13.4	13.0	21.3	23.4
Growth/assimilation (NP_n/A_n)	11	23.6	21.9	35.5	39.1
Surviving adults/growth		30.5	26.1	26.5	24.8
Mortality/growth		69.5	73.9	73.5	75.2
Respiration/ingestion (R_n/I_n)	3-0	42.8	45.9	37.7	35.4
Respiration/assimilation (R_n/A_n)	10	75.3	77.1	62.9	60.0
Assimilation/ingestion (A_n/I_n)	1-0	56.8	59.5	60.0	59.8

[a] Kozlovsky's classification system.

ingested (22%) and assimilated (36%) into growth. Of the total growth only 27% was channeled to adult populations in both springs. The highest percentage of population growth channeled to surviving adults was in spring 6 in 1965. Over 70% of the annual increment of population growth in both springs was lost to mortality and an estimated 90% of this mortality was attributable to larval export.

Teal (1957) noted that the amount of metabolizable energy required for maintenance averaged 75% for all herbivores in the Root Spring community, and Golley (1960) estimated that *Microtus* populations put 68% of assimilated food into maintenance. *Hedriodiscus* populations in spring 6 respired about 76% of the metabolizable energy intake whereas populations in spring 4 respired only 61%. A partial explanation for this difference may be related to observable differences in their diurnal behaviour patterns. During the day larvae in spring 6 generally remain in contact with warm water (35 C) at the margin of the spring, usually hidden beneath the *Oedogonium* mat with their respiratory siphons at the surface for oxygen intake. Thus the larvae are in contact with warm water at all times. Larvae in spring 4 cannot tolerate the 47 C temperature beneath the *Schizothix–Phormidium* mat and are found beneath decaying timbers or under overhanging grass that surrounds the spring pool and lies adjacent to the stream. The temperature at these localities never exceeds 27 C. This difference in diurnal behaviour may explain differences between the two populations relative to the proportion of metabolizable energy put into growth and respiration. It would be reasonable to expect higher maintenance costs in those populations spending 24 hr in contact with warm water in spring 6 as opposed to those that during the day spend a large portion of time in cooler areas. The observed differences between oxygen consumption at 25 versus 35 C, obtained from respiration measurements, adds validity to this interpretation.

Both populations possess similar assimilation efficiencies, averaging 60%, but larvae in spring 4 put a greater portion of this energy into growth than respiration. This accounts for higher growth rates observed among instars in this spring.

Population regulation

When populations were sampled in 1965 and 1966 to determine seasonal variation in numbers, biomass, and energy flow, very little seasonal fluctuation in any of these parameters was evident between springs or between years. In 1964, however, two fortuitous events, namely trampling by elk and decomposition of parboiled elk, illustrated the response of these populations to environmental disturbance. It is apparent that the energy content or nutritional quality of food intake regulates larval growth rates, and hence the resultant number of generations per year. It is clear from this study that *Hedriodiscus* populations inhabiting the Ohanapecosh Hot Springs are limited by food quality to two generations per year or two periods of reproduction per year. It is quite likely that a steady diet of 5000 + calories/ash free gram during the growing season will increase the generations per year by one with a resultant increase in reproductive output.

There is no limitation in quantity of food, as food is always present in superabundance and is not appreciably removed during the summer by grazing larvae. The caloric content of the algal mats changes seasonally but the variation does not deviate significantly ($P = 0.05$) from the mean summer caloric value (Stockner 1968b).

Only about 1% of the total net primary production in both springs is ingested by *Hedriodiscus* populations. Similar data from other energy studies are within this range (Slobodkin et al. 1967).

These data lend credence to the argument of Hairston et al. (1960) of a general lack of food limitation of populations of terrestrial herbivores. Clearly, in certain situations this also applies to herbivores in the aquatic environment.

It is apparent that the most important factors regulating biomass and numbers of *Hedriodiscus* populations in the Ohanapecosh Hot Springs are export mortality and spider predation of emerging adults. The average export of larvae during the summer of 1966 was $0.6/m^2 \cdot 24$ hr. This value when compared with field population census data accounts for approximately 90% of population mortality between sampling periods. This "leakage" undoubtedly prevents larval populations from expanding to the point where other factors would operate with increasing efficiency. The predaceous spiders *Pirata* and *Tetragnatha* prey upon newly emerged adults and are responsible for the sparse adult populations observed at Ohanapecosh. Though accounting for far less of total mortality, spider predation comes at a very crucial time in the life history and may in one sense be a more important regulating factor than export. For example if fourth instar pupating larvae were immune to predation, approximately 14 adults would emerge/m^2, and if one assumes half are females, then the mat would receive an inoculum of around 1155 eggs/m^2 as opposed to the observed mean number of 246/m^2.

Two physical factors of importance in influencing the development of communities of both springs are temperature and current velocity. The effects of high temperature on the organization and structure can be seen by reexamination of the food-web diagrams (Fig. 2). The obvious inverse relation between community structure (diversity) and temperature is well documented (Stockner 1967b, 1968a, b; Winterbourn and Brown 1967). Current velocity or "flow" has also affected the number and type of species capable of adapting to the thermal environment. The effectiveness of current in reducing larval population density has already been discussed. Tilly (1968) in his study of Cone Spring considers this cold spring ecosystem to be maintained in a state of immaturity by the influence of "flow" and low temperature. A similar state of immaturity exists at the Ohanapecosh springs and these two physical factors (with the exception of high instead of low temperature) play an equally important role in maintaining this condition at Ohanapecosh.

The Community Energy Budget

The mean annual income and expenditure of energy for springs 4 and 6 were compared from estimates obtained in 1966 (Table 9). Specific sources and amounts of energy income and expenditure were dissimilar between springs. Reasons for these differences are discussed below.

Energy income

Two of the major sources of income to the springs are energy fixed by primary producers and energy contained in plant debris that enters the springs in autumn. Other possible sources of less importance are: (*a*) immigration of aquatic insects; and (*b*) energy contained in animal corpses that have been parboiled.

The amount of energy contributed by photosynthesis in spring 6 was calculated from measurements of diurnal oxygen change from April to September and this amounted to 4016 Kcal/m^2·year. Direct field measurements of primary production were not obtained from spring 4, mainly because the 47 C water temperature prevented reliable estimates of diurnal oxygen change. The algal species in spring 3 (37 C) are identical to those in spring 4, and both springs also have a similar water chemistry (Stockner 1968b). Thus, in the absence of direct measurements, the most reasonable approximation of annual primary energy income to spring 4 is the integrated value obtained from spring 3, 4607 Kcal/m^2·year. This value is likely an underestimation, for there appears to be a direct, positive relation between production and temperature in thermophilic Cyanophyta in a temperature gradient from 30 to 50 C (Brock and Brock 1967; Brock 1967). Of the estimated total energy income, 95% was derived from algal primary production in spring 4 and 89% in spring 6.

Income attributed to shedding of leaves in fall by the maples *Acer macrophyllum* and *A. circinatum* was estimated for 1965 and 1966. In 1965, 84.2 g/m^2 entered the pool and stream margins of spring 6 and this amounted to an annual income of 414 Kcal/m^2·year. In 1966 slightly higher values were obtained: 89.1 g or 439 Kcal/m^2·year. The income of primarily *A. circinatum* to spring 4 was considerably less than for spring 6 and the mean value for both years was 38.6 g/m^2·year or 184 Kcal/m^2·year. Leaves that collected in the streams and pools were used as substrate for algal growth and were

TABLE 9. Mean annual balance sheet of organic matter 1966, Ohanapecosh Hot Springs, Mount Rainier National Park.

Source	Kcal/ m^2·year	% of total
Spring 4		
Income		
Primary production	4607	95.7
Organic debris	184	3.8
Immigration of adult terrestrial insects	22	0.5
Total	4813	
Expenditures		
Community respiration	1158	24.0
Export	1461	30.3
Emigration of adult insects	13	0.4
Deposition[a]	2181	45.3
Total	4813	
Spring 6		
Income		
Primary production	4016	89.1
Organic debris	439	9.5
Immigration of adult terrestrial insects	62	1.4
Total	4517	
Expenditures		
Community respiration	1968	43.7
Export	1047	23.2
Emigration of adult insects	78	1.8
Deposition[a]	1424	31.3
Total	4517	

[a] Values of deposition obtained by difference.

quickly covered with a thin algal coating. In addition to providing substrate they tended to shade out algal mat beneath them, which consequently decomposed and gradually sloughed.

Leaves were examined at 2-week intervals to determine whether they were being used as a food source by aquatic invertebrates. After several weeks of immersion there was no sign of their being used as a food source by *Hedriodiscus* or any other invertebrates. Most leaves are deposited around the margin of the pools and streams and are quickly decomposed by bacteria. This is particularly evident in spring 6 where, within a month or two, little evidence remains of any massive autumn leaf fall. Mineralization occurs very rapidly in hot spring pools and shallow streams where autochthonous and allochthonous material can accumulate (for further discussion of the sediments of spring 6, see Stockner 1968a).

More organic debris (primarily leaves of *Acer*) entered spring 6 than spring 4. This is due to the close proximity of spring 6 to bordering *A. macrophyllum* trees. A more extensively developed overhanging vegetation of grasses and sedges surrounds spring 6, and this feature accounted for differences between springs in energy income from terrestrial insects.

Immigration of aquatic insects from other habitats was never observed and cannot be considered a significant source of energy income. As already briefly discussed, terrestrial insects that enter the streams are quickly exported and hence do not contribute appreciably to energy income. In quiet spring pools, however, adult insects, primarily dipterans, could often be observed floating on the surface. Corpses of amphibians and reptiles were also occasionally found, chiefly during the spring and fall periods. These corpses, together with molted exoskeleton material from *Hedriodiscus* and odonates, contributed some energy to the springs, estimated to be 22 and 62 Kcal/m^2·year for springs 4 and 6, respectively. *Hedriodiscus* exoskeleton material cannot be considered income since the energy required for synthesis of this material was derived from the spring. Thus it can, in a sense, be considered "interest" payment on the original energy investment. Terrestrial insect import, though a negligible source of energy income, was particularly heavy on warm, windless days in both May and September.

Energy loss

The loss of energy from springs 4 and 6 occurred in several ways: (*a*) particulate and dissolved organic matter, (*b*) emergence of adult aquatic insects (Diptera, Odonota), (*c*) emigration of larvae, (*d*) loss of larvae and adults to predators, and (*e*) respiration.

The loss of particulate matter in the form of algal fragments has been given attention elsewhere (Stockner 1968b). The dissolved organic component represents a significant loss that is difficult to assess in relation to its effects on the spring community. Utilization of this energy source by microbenthic components of the community and the actual measurement of this loss was not investigated.

The loss of organic matter in the form of adult insects has been briefly discussed and represents a negligible loss in relation to other more important items of the energy budget. Similarly, active emigration of aquatic insects, most notably *Hedriodiscus*, is a negligible factor. Organisms swept from the algal mats represent an energy loss from the system (export), whereas other animals of terrestrial origin that fall into the warm water, succumb, and are then swept away, do not.

Community respiration and export together represented the major loss of energy from the springs, totaling 54% for spring 4 and 67% for spring 6. The export of energy from spring 4 was higher due to both a higher current velocity and the nature of the blue-green mat morphology (Stockner 1968b). Lack of a noticeable algal "mat" and a more moderate current reduced the total algal export from spring 6.

Community respiration values were considerably higher in spring 6, due to the presence of other herbivores besides *Hedriodiscus*, and to increased bacterial respiration. The lower values obtained from spring 4 are not surprising since *Hedriodiscus* is the only herbivore and bacterial activity is negligible when compared with spring 6 (Stockner 1967a). The energy leaving the springs in the form of adult insects amounted to 78 Kcal/m^2 for spring 6 and 13 Kcal/m^2 for spring 4. This discrepancy is attributable to differences in community structure between springs.

Estimates of amounts of energy lost by deposition were obtained by difference, and amounted to 45% of the total annual expenditure in spring 4, but only 31% in spring 6. These amounts of energy deposition are not unrealistic nor are they surprising since it is obvious that the springs are continually building up and have been for hundreds of years. The role of algae in travertine deposition has been known for some time (see Allen and Day 1935). It is clear from observation alone that large amounts of organic matter are continually lost during the gradual process of travertine formation and deposition. Thus, this estimate, though high in terms of the total budget, does not seem unrealistic in the light of present information.

Efficiency of energy fixation and conversion

The efficiency with which plants convert solar radiation into chemical energy is referred to as photosynthetic efficiency. There has been much confusion over the use of this ratio because of the imprecisely stated values of both numerator and denominator. The definition most acceptable to ecologists is defined as net production divided by the total amount of visible light reaching the plant (4000–7000 Å). The calculated photosynthetic efficiency at Ohanapecosh of 1.06 is well within the range of known published values. Of the yearly total organic production, 0.5% is channeled through the herbivore *Hedriodiscus* in spring 4 and 0.7% in spring 6. This is equivalent to ratio EE57, In/NP_{n-1} or "consumption efficiency" (Koslovsky 1968). If NP_{n-1} is corrected for amounts lost by export, i.e. In/NP_{n-1} – $Export_{n-1}$, then the efficiencies increase to 5 and 6%, respectively. It is clear from this ratio that only a small portion of available food is passed to the major consumer, *Hedriodiscus*. Both spring 4 and 6 are clearly export systems, fixing far more energy than is consumed.

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NOTES

Differential Growth Rate in the Tail of the Pacific Big Skate, *Raja binoculata*

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FORD, P. 1971. Differential growth rate in the tail of the Pacific big skate, *Raja binoculata*. J. Fish. Res. Bd. Canada 28: 95–98.

Over a period of years the egg cases of *Raja binoculata* Girard were collected from waters in the Strait of Georgia, British Columbia, and measurements of the total body lengths and tail lengths were recorded. From these data an hypothesis was formulated suggesting that the relative growth rate of the tail to the growth of embryos is related to the mode of respiration utilized at a given time during development.

The method of respiration employed by embryos of elasmobranchs follows a chronological sequence of integumentary, external gill filament, and spiracular–branchial respiration. To determine whether this hypothesis was tenable the data were subjected to a statistical analysis. A linear regression on the logarithms of the data was performed for caudal lengths below and above 8.5 cm. In each instance the probability that the resulting slope was not zero was less than 0.0001. Further, an analysis of covariance was performed that indicated that the two slopes were significantly different with a probability of less than 0.005.

It would seem reasonable on the basis of the above analysis to assume that the tail length–respiration relation is a tenable hypothesis.

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THE problems of relative growth have interested biologists since the work of Thompson (1917) and subsequently of Huxley (1932). De Beer (1951) reviewed the significance of growth rates in ontogeny and phylogeny and stressed the importance of heterochrony.

The Pacific big skate (*Raja binoculata* Girard) is an oviparous elasmobranch found on the Pacific coast of North America and was first taken in British Columbia waters in 1880 (Clemens and Wilby 1961). Egg cases were taken for studies on the development of this species (McConnachie and Ford 1965, 1966) in the Strait of Georgia at Roberts Bank and off Comox on Vancouver Island, B.C. The cases contain from one to eight eggs and all stages may be found at any time of the year. It is presumed that the period of gestation of the eggs is in excess of 12 months.

The purpose of this study is to confirm the hypothesis that the relative growth of the tail of *R. binoculata* is related to the respiratory mechanisms employed by the embryos.

Materials and methods — Skate egg cases were collected in the Strait of Georgia from the summer of 1966 to the summer of 1969 by M.V. *Investigator II* of the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C. In all, 86 individuals were measured and their rostral–caudal and cloacal–caudal lengths were recorded.

Observations — It was apparent that the tail of the measured individuals grew in length relatively faster than the total overall length (Fig. 1). A comparison was made between those individuals in which respiration was assumed to be either by diffusion through the integument, by gill filaments, or by branchial gills. A difference was shown in growth rate in those individuals using branchial respiration. When rostral–caudal lengths are plotted against caudal lengths on a log/log curve (Fig. 2), it can be seen that at the time of the initiation of branchial–spiracular respiration and the cessation of tail activity, the slope of the curve undergoes an abrupt change. That is, the data above and below the caudal length of 8.5 cm differ markedly from one another. To verify the biological validity of these observations the data were subjected to a statistical analysis.

Statistical analysis — To test the hypothesis that there is a linear relation between the logarithms of the two measurements recorded, we first performed a linear regression on the logarithms of

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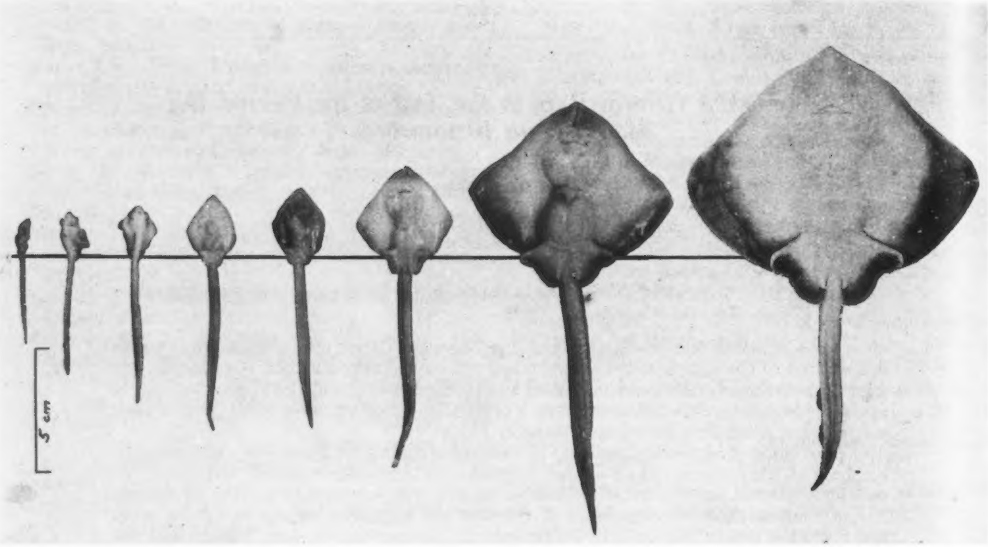

FIG. 1. A representative series of developing embryos seen from the ventral surface with the yolk sac removed. The final embryo shows a marked shortening due to resorption. (Rostral–cloacal above bar, cloacal–caudal below.)

the data for caudal lengths below and above 8.5 cm. In each instance the probability that the resulting slope of the line was not zero was less than 0.0001. For the data below 8.5 cm we have the equation

$$y = 1.7501\,x^{0.8615},$$

which at 8.5 cm gives $y = 11.06$; for the data above 8.5 cm we have the equation

$$y = 0.2167\,x^{1.8680},$$

which at 8.5 cm gives $y = 11.8$.

Secondly, to establish that the slopes of the logarithms of the data for caudal lengths below and above 8.5 cm were different, an analysis of covariance was performed. This analysis indicated that the two slopes were significantly different with a probability of less than 0.005.

Discussion — During the development of fish embryos, external gills are found in several species to serve not only respiration but also the absorption of food substances taken from the maternal uterus and probably excretion and ion balance. In Clemens (1895) these external gills are described as being divided into two groups having origins either in the endoderm or the ectoderm. In the elasmobranchs these filaments issue from the branchial slits or the opercular orifice (Bertin 1958). Each filament is irrigated by a vascular loop between the afferent and efferent arteries of the corresponding internal gill. Their number and length vary greatly from group to group.

The early development of the oviparous species *Mustelus canis* was described by Te Winkel (1950); she later made special reference to the early periods of gestation (1963). In addition, in her work on development of the viviparous species *Squalus acanthias* on embryonic nutrition (1943), Te Winkel also refers to the condition of the external gill filaments.

The branchial filaments are passive structures, their motion caused by undulating movements of the tail, which is continuously moving from the time of its first appearance, that is before the appearance of gill filaments, until the onset of branchial–spiracular respiration. One may presume that the tail also keeps the embryonic milieu in circulation.

In association with these activities the tail grows at an enormous rate in relation to the growth of the body, achieving a maximum relative length in our individuals of 8.5 cm. Prior to the onset of branchial-spiracular respiration, and during the period of tail activity, no other muscular movements of the embryo can be observed, but on the cessation of tail activity, undulating movements of the large pectoral fins can be seen and the embryos swim from the egg cases in this manner.

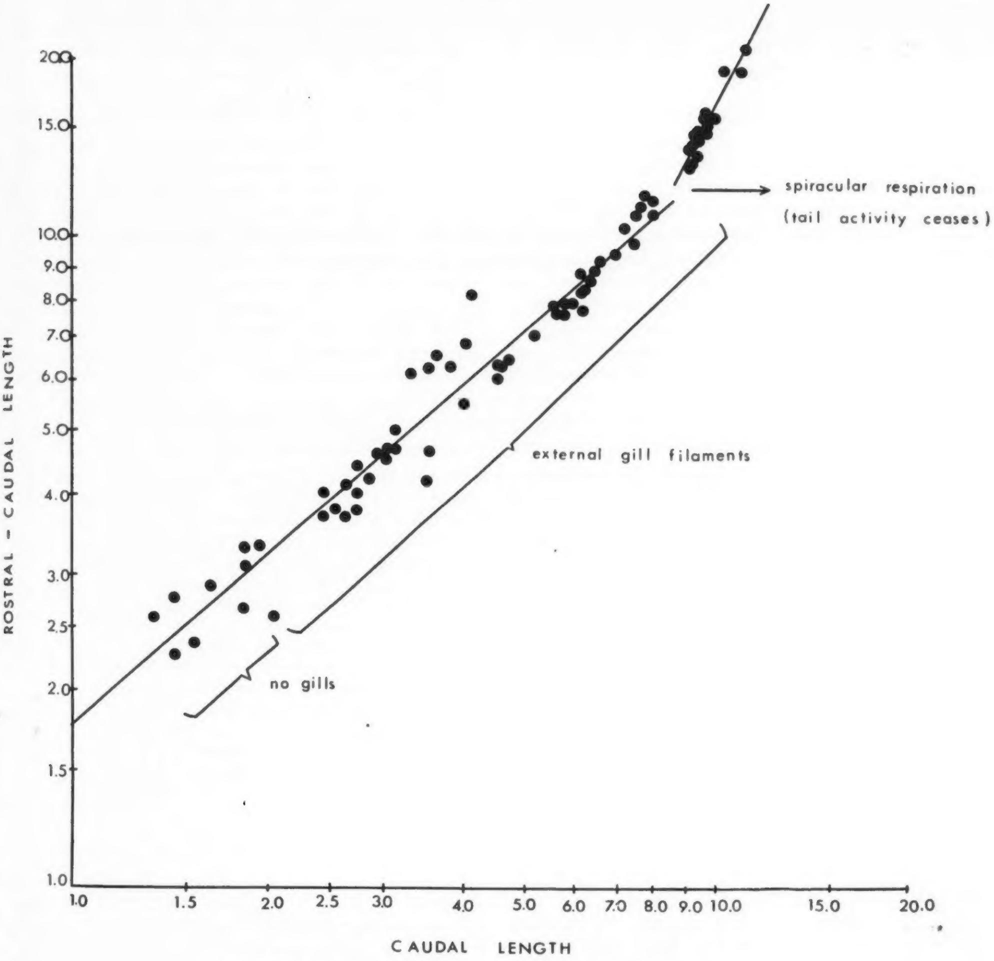

FIG. 2. Log/log plot of rostral–caudal and caudal lengths.

After hatching, the achievement of the final reduction of the length of the tail is by means of resorption (Fig. 1). We have not considered this phenomenon in our data.

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Sternal Spines as a Characteristic for Differentiating between Females of some Pandalidae

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A life-history study of four commercially important species of pandalid shrimps in Alaska shows that with the exception of one species, a morphological characteristic is possessed that is useful in estimating age and survival of certain females. *Pandalus borealis*, *P. goniurus*, *P. hypsinotus*, and *Pandalopsis dispar* possess a series of small spines located on the median ventral surface of the abdomen or sternites. With the exception of *P. dispar*, the sternal spines are present throughout the postlarval life of the shrimp until the first molt into breeding dress as a female. Only females that are sexually mature and are very near egg extrusion undergo this molt. At this time, the spines are either completely lost or reduced to minute protuberances. Between spawning seasons, females that extruded eggs during a prior spawning season can be easily identified as those that do not possess abdominal spines or possess only minute protuberances. Therefore, females that possess spines during any time of year are those that have not previously spawned. This characteristic is especially useful during the period between successive spawning seasons when it can be an effective tool in determining the number of females surviving one or more spawnings. Age and survival of pandalids is usually estimated through analysis of length-frequency ranges. This is often difficult with respect to females because of overlapping length ranges between age classes. Sternal spines may be useful in studies of other pandalids and families of shrimp.

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THE age of pandalid shrimps is usually determined through establishment and analysis of length-frequency ranges for each age class. The length-frequency ranges of female shrimps that are approaching sexual maturity often overlap the length ranges occupied by older age-groups of previously spawned females. This overlap makes it difficult to distinguish between these two groups with any degree of certainty. However, differentiation of these females is necessary for accurate determinations of age-class structure and survival rates. The purpose of this article is to describe, for certain species of pandalids, a method by which previously spawned females may be distinguished from those females not having spawned previously.

Presently the life histories of four commercially important species of pandalid shrimps from Alaska, *Pandalus borealis* Krøyer, *P. goniurus* Stimpson, *P. hypsinotus* Brandt, and *Pandalopsis dispar* Rathbun, are being studied. These pandalids typically exhibit protandric hermaphroditism where sexual maturity as a male is reached first, followed by a transition (sex change), after which the remainder of life is spent as a female. Exceptions to protandric hermaphroditism are noted in the cases of primary and secondary female development where male tendencies are repressed early in life, and maturity is reached only as a female.

Results — The pandalids in this study possess a morphological characteristic that, with the exception of one species discussed later in this article, is useful in determining if a female has spawned previously. Once this can be determined, certain inferences may be more accurately drawn with respect to age and survival between successive

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spawning seasons. The characteristic consists of a series of spines located on the midventral surface of the first four abdominal segments or sternites (Fig. 1). These sternal spines are present throughout the postlarval life of the shrimp until the first molt into breeding dress as a female. Only females that are sexually mature and are very near egg extrusion undergo this molt. At this time, the spines are either completely lost or reduced to minute protuberances. Between spawning seasons, females that extruded eggs during a prior spawning season can be easily identified as those that do not possess abdominal spines or possess only minute protuberances. Therefore, females that possess spines during any time of year are those that have not previously spawned.

Coutiere (1899) observed sternal spines in Alpheidae, Nouvel and Holthuis (1957) in one species of Processidae, and Holthuis (1946) in Stenopodidae. The latter author described them as "median spines on the ventral surface of the abdominal segments." The sternal spines of pandalids in the current study occur medially at the junction of the sternites of the first four abdominal segments (Fig. 1). This junction occurs as a ridge between each sternite and forms a transverse connection between the bases of each individual pair of pleopods. Paired spines are present on the sternal ridges of the first three abdominal segments. A single spine occurs on the sternal ridge of the fourth segment and a small laterally compressed keel or carina extends anteriorly from the sternal ridge of the fifth segment (Fig. 1). The spines on the first three sternal ridges are the most prominent; those located posteriorly become progressively smaller. The single spine on the fourth sternal ridge is smallest and can be difficult to detect as it may persist as a small blunt protrusion or minute protuberance when the paired spines are prominent. The carina of the fifth sternite is also somewhat variable in form. Thus, the single spine on the fourth sternal ridge and the carina on the fifth sternite are less reliable in distinguishing between females than the paired spines of the first three sternites.

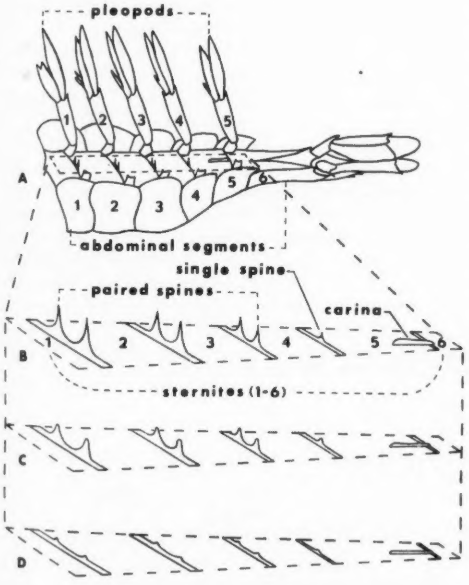

FIG. 1. A: Diagrammatic ventral view of the abdomen of a pandalid shrimp showing the location of sternal spines. B–D: Expanded ventral views of sternal spines in prominent, reduced, and remnant stages respectively.

In this study, the presence or absence of sternal spines has been recorded since March 1969. Several thousand specimens of *P. borealis* have been examined in the laboratory and supplemented with extensive field observations. Fewer observations have been made with respect to *P. goniurus*, *P. hypsinotus*, and *P. dispar*, but several hundred specimens of each species were examined in the laboratory and field. With the exception of *P. dispar*, all observations indicate that the loss of sternal spines coincides with the individual's first molt as a female into breeding dress. Thus, many newly transformed females are present between successive spawning periods that do not have spines. In this species, sternal spines appear not to be reliable for distinguishing between females that have and have not previously extruded eggs.

All males and most transitional *P. borealis*, *P. goniurus*, and *P. hypsinotus* possess prominent spines (Fig. 1B). Occasionally during late stage transition, a slight reduction in the size of spines occurs. In these instances, the sharp tips of the spines appear to become slightly blunted (Fig. 1C). Some females, after assuming breeding dress, commonly retain minute protuberances or remnants in place of spines (Fig. 1D). The differences in appearance between prominent spines, reduced spines, and remnant spines are distinct (Fig. 1B–D).

During the period between successive spawning seasons, using *P. borealis* as an example, two groups of females are present (Table 1). One group of newly transformed females has prominent spines and the other previously spawned group is without spines or possesses only remnants. Spawning is obligatory upon reaching sexual maturity; therefore, as the spawning season progresses, the percentage of females with spines gradually diminishes. Finally,

TABLE 1. The loss of sternal spines in *Pandalus borealis* females in relation to the progression of spawning.

Month	No. shrimp	No. females	% of females: With spines	% of females: Without spines	% of females: Ovigerous or in breeding dress
1969					
Aug.	5885	1050	41.2	58.8	0.0
Sept.	4362	857	51.1	48.9	0.9
Oct.	4805	1720	9.2	90.8	79.0
Nov.	6877	2178	2.6	97.4	93.6
Dec.	2646	702	3.1	96.9	95.4
1970					
Jan.	1424	340	0.0	100.0	95.4
Feb.	1567	410	0.0	100.0	100.0
Mar.	1534	753	0.0	100.0	99.7
Apr.	2534	976	0.0	100.0	84.6
May	2964	995	0.0	100.0	58.8

as the percentage of ovigerous females nears 100, a corresponding increase in percentage of females without spines occurs. Egg hatching usually begins in late March and most hatching is completed by early May. This is reflected by the decreasing percentages of females that are ovigerous or in breeding dress. By June a few early transforming individuals complete sex change, and a corresponding increase in the percentage of females with spines would be expected.

Discussion — The presence or absence of sternal spines in certain pandalids is a useful morphological characteristic for distinguishing between previously spawned females and those that have not previously spawned. This characteristic is especially useful during the period between successive spawning seasons when it can be an effective tool in determining the number of females surviving one or more spawnings.

Whether this characteristic is useful in studies of other pandalids is not known. Examinations of at least 50 specimens of *Pandalus platyceros* Brandt, *P. jordani* Rathbun, and *P. danae* Stimpson indicate that a similar relation of spines may be true. All males and transitionals observed had spines, but ovigerous females did not. Casual observations of several species belonging to families Hippolydidae and Crangonidae indicated that males but not ovigerous females possess spines. Hippolytids exhibited paired spines on the sternal ridges of the first three abdominal segments, whereas crangonids exhibited single spines on the sternal ridges of the first four abdominal segments.

Acknowledgments — I extend a special thanks to project technician Mr D. Jobson, who originally questioned if the presence or absence of sternal spines could be of some biological value.

Thanks are also due to D. Petersen, P. Jackson, J. McMullen, E. Haynes, and D. Gotschall who offered helpful suggestions in the preparation of the manuscript.

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A Device for Rapid Removal of Embryos from Fish Eggs

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MACKELVIE, R. M. 1971. A device for rapid removal of embryos from fish eggs. J. Fish. Res. Bd. Canada 28: 100–102.

An inexpensive and easily constructed device is described whereby embryos were removed from fish eggs for the study of viral multiplication.

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THE device was designed to permit the easy opening of brook trout (*Salvelinus fontinalis*) eggs for the removal of their embryos, which were required for replication studies of the infectious pancreatic necrosis virus (MacKelvie and Artsob 1969). Essentially it is a means whereby the egg is held while a segment of it, distal to the embryo, is removed by cutting through the zona radiata and the underlying vitelline membrane. The embryo may subsequently be removed by pasteur pipette.

The device (Fig. 1) consists of two rectangles of $\frac{1}{4}$-inch-thick leucite; one piece, the base, measures 3 × 1$\frac{1}{4}$ inches and the other, which forms the body, measures 2 × 1 inch. On the face, midway on the long edge of the base and approximately $\frac{1}{4}$ inch in from it, a depression $\frac{3}{64}$ inch deep was made with a $\frac{3}{16}$ inch roundnose end mill. The same end mill was used to drill a hole in the face of the body piece in the same relative position as the depression in the base. The depth of this hole was such that an average size brook trout egg was snugly enclosed in the cavity formed by juxtaposing the base depression with the body cavity when the two pieces of leucite were spaced apart the thickness of a safety razor blade. Two fragments of razor blade glued to the base at the appropriate places act as spacers to allow insertion of the cutting blade between the base and the body. A vent, $\frac{1}{64}$ inch in diameter, was drilled to the exterior through the roof of the body cavity to prevent the exertion of undue pressure upon the egg when positioned in the assembly.

The procedure for use is as follows: set the base on the stage of a dissecting microscope with the depression centered in the field of vision. Into the depression place one drop of phosphate buffered saline and then an egg that has been blotted dry. Using a spatula, orientate the egg so that the embryo is uppermost. Almost invariably the embryo will assume a tail to head configuration above its yolk, which greatly facilitates its total enclosure within the cavity of the body piece when this is gently lowered into position. With the base and body pieces held firmly together the device is removed from the microscope stage and a razor blade is inserted into the space between them (Fig. 2). It is necessary to make the cut with a fairly brisk motion for any hesitation will cause the embryo to thrash with a consequent risk of its mutilation. After the cut has been made the razor blade is held firmly against the body piece as the base is slid away; the blade and body piece are inverted and finally the blade is carefully withdrawn to leave the body piece with the exposed embryo within the cavity. The addition of a drop of phosphate buffered saline facilitates collection of the embryo headfirst into a suitable pipette. With

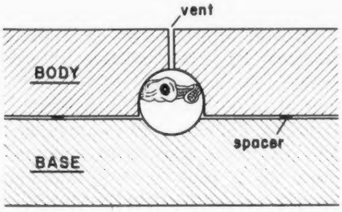

FIG. 1. Cross-sectional diagram of cutting device with a 3-dimensional embryo sketched in situ.

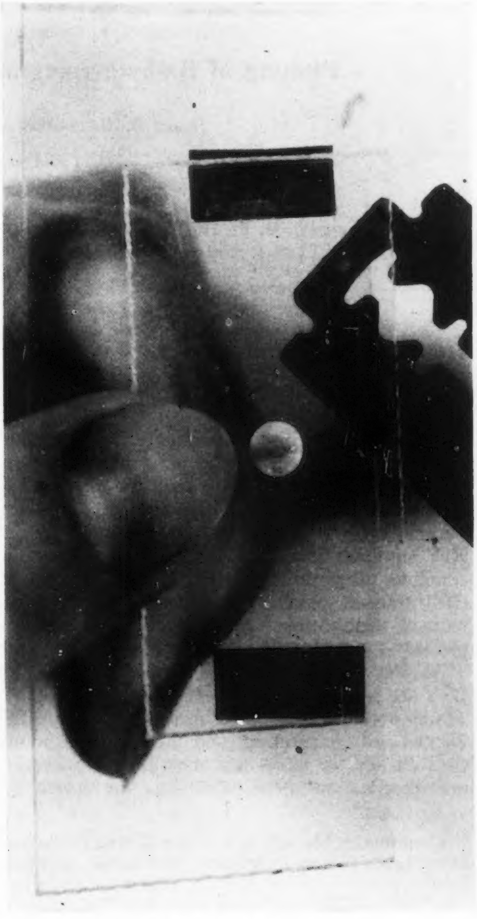

FIG. 2. The device in operation.

experience the entire operation can be performed in well under 1 min.

The product of this procedure appeared to be particularly suitable for studies on infectious pancreatic necrosis virus multiplication. The heart continued to beat for several days when the embryo was maintained in a nutrient medium, which, in conjunction with the severed vitelline blood vessels probably aided dissemination of added virus throughout the body of the embryo. Several other uses for these excised embryos may be readily suggested, including, for example, provision of tissue for cell culture initiation, histological (and histopathological) studies, and various biochemical investigations. The primary advantage in the use of this device appears to lie in the speed and ease with which it provides a supply of physiologically sound embryos for in vitro studies. The design can be modified for use with eggs of other fish species merely by varying the size of the base and body depressions.

Acknowledgments — The technical assistance of Mr R. C. Edmonds is gratefully acknowledged.

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Plotting of Bathythermograph Transect Data on a Printer[1]

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Reynolds, J. B., and D. R. McLain. 1971. Plotting of bathythermograph transect data on a printer. J. Fish. Res. Bd. Canada 28: 102–103.

A program for plotting bathythermograph transect data on a computer (IBM 1130) printer is available from the Great Lakes Fishery Laboratory. Temperature values are printed in positions proportional to their depths and distances from shore. Contour lines are drawn manually through the plotted points.

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Contoured plots are often desired as the final output from processing of limnological data. Many limnological computer programs, however, either provide only tabular listings of the data or, when they do provide contoured plots, require a special X–Y plotting device. In this note we describe a computer program for presenting bathythermograph (BT) transect data in a manner convenient for manual contouring. A conventional computer printer is used as the output device.

At the Great Lakes Fishery Laboratory, all BT's are calibrated in units of feet and degrees C. Traces obtained from BT's are digitized manually. Depths and temperatures and the inflection points (i.e., changes in trace direction) and at isotherms are recorded on forms. The data are stored on punch cards as temperature–depth (T–D) pairs. The number of T–D pairs representing a trace, and, where applicable, the position of the T–D pairs representing the upper and lower limits of the thermocline, are also stored.

Three formats of punched cards are read in the computer program as input data. The first (a single card) provides header information for a title printed on the plot; the second provides the distances between BT stations in statute miles; and the third contains the BT data (i.e., T–D pairs, number of T–D pairs, and the limits of the thermocline) in a format similar to that of the Bathythermograph Log published by the National Oceanographic Data Center.

The computer reads the T–D pair data for the first BT station, and, because of space limitations, prints only the units and tenths positions of the temperature values (Fig. 1). For example, at BT station 01, surface temperature is 13.0 C, but only the 3 and 0 are printed at the surface position (encircled in Fig. 1); at the bottom (30 ft), where

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[1] Contribution No. 429, U.S. Fish and Wildlife Service, Great Lakes Fishery Laboratory, Ann Arbor, Michigan 48107.

Printed in Canada (J1790)

the temperature is 10.9 C, only the 0 and 9 are printed. Temperature values are spaced according to their respective depths, starting with the surface value on the right side of the printer page. The reading and printing procedure is repeated for all BT stations in a transect. The distance between stations on the paper is proportional to the actual distance between stations. New page skips are suppressed as the paper advances to give uninterrupted plotting along the distance axis. The BT cast number (BT), distance from the starting point of the transect (MLS), surface temperature (SFC TMP), and bottom temperature (BOT TMP) are printed at each station position (Fig. 1).

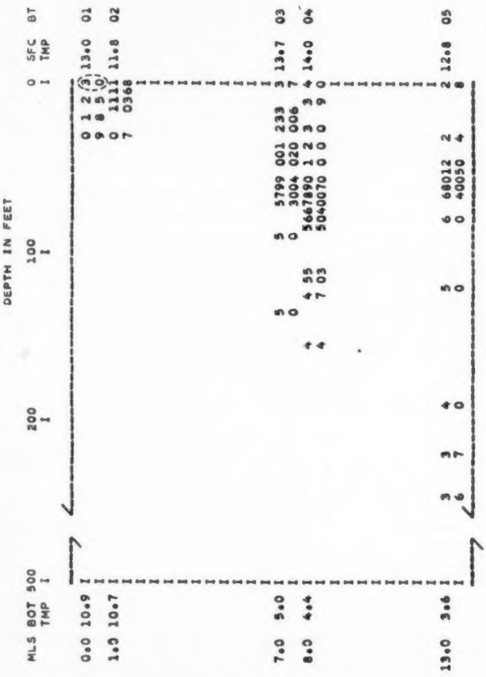

FIG. 1. Printer plot of BT data from a transect in southern Lake Michigan, 1968. Distance in miles, depth in feet, and temperature in degrees C. The characters printed are units and tenths of temperature values at depth of observation. The units characters are at station location along the distance axis.

After the data for all stations are printed, the paper is rotated 90° to bring the right side (i.e., surface) to the top for normal perspective. The isotherm contours are then drawn in manually (Fig. 2). If the depths and temperatures of most of the isotherms were recorded when the BT slide was originally digitized, contouring is very simple; if only a few isotherms were recorded, manual interpolation is required.

The BT plotting program has been written in FORTRAN for the IBM 1130 computing system (8K, single disk) at the Great Lakes Fishery Laboratory. A documentation of the card formats and plotting program is available from the authors, as are copies of the program in punch-card form. The documentation includes suggested modifications of the program for use with other card formats or units of measurement.

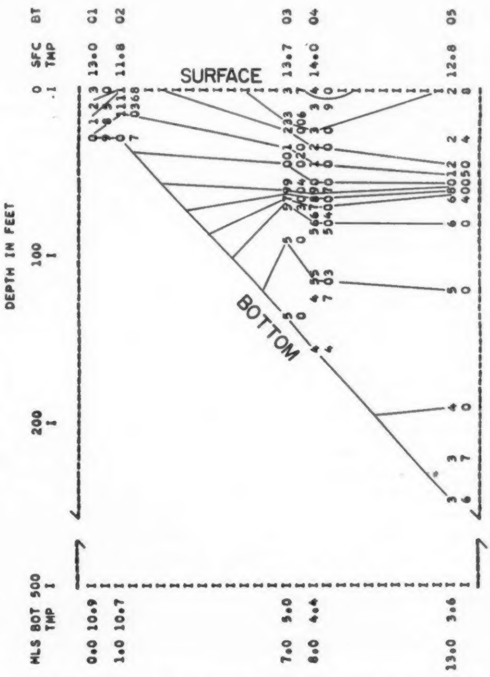

FIG. 2. Manual contouring of the printer plot. Figure rotated 90° for normal perspective.

A Proteolytic Pseudomonad from Skin Lesions of Rainbow Trout (*Salmo gairdneri*). III. Morphological Studies

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LI, M. F., AND G. S. TRAXLER. 1971. A proteolytic pseudomonad from skin lesions of rainbow trout (*Salmo gairdneri*). III. Morphological studies. J. Fish. Res. Bd. Canada 28: 104–105.

The morphology of a pseudomonad isolated from diseased rainbow trout was examined in detail. The organism was lophotrichous, and the fine structure was morphologically typical of most marine species of *Pseudomonas*.

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THE involvement in fish diseases of *Pseudomonas fluorescens* and closely related organisms has been reported repeatedly. Our previous communications described the pathogenicity of a pseudomonad isolated from diseased rainbow trout, and certain properties of its powerful extracellular proteinase (Li and Flemming 1967; Li and Jordan 1968). The morphology of this pseudomonad pathogenic for fish has now been investigated extensively by us and in this report the attempt to determine the extent of morphological variation between this pathogen and known aquatic pseudomonads is described.

Materials and methods — Stock cultures were maintained on Trypticase soy agar at 4 C. For negative contrast staining, organisms from colonies of 24-hr cultures were suspended in 1% aqueous potassium phosphotungstate solution at *p*H 7.0 (Richter and Kress 1967). Droplets of these suspensions were spread on grids coated with Formvar and drained. Organisms grown in Trypticase soy broth were used for examination of ultrathin sections. One of two alternate methods was used to fix the cells before sectioning. In method A, the cells were fixed with osmium tetroxide (Kellenberger et al. 1958). In method B, a double fixation of the loose cell pellets with glutaraldehyde and osmium tetroxide in phosphate buffer was employed, and the sections were stained with uranyl acetate followed by Millonig lead staining (Li and Traxler 1970). Sections were cut with glass knives on an LKB ultramicrotome. The prepared specimens were examined in a Hitachi HS7S electron microscope.

Observations and discussion — We had suggested previously on the basis of optical microscope observations that this organism appeared to have a single flagellum. Obtaining accurate pictures of flagella by light microscopy is, however, extremely difficult (Hodgkiss 1960). Examination of the bacteria by electron microscopy in contrast revealed a flagellar tuft located at one pole of the cell (Fig. 1). This observation agrees with the statement by Bullock and his co-workers (Bullock et al. 1965) that most pseudomonads isolated from diseased fish were lophotrichous.

Thin sections prepared by the Kellenberger procedure (Fig. 2) show the undulant cell wall, which was separated from the cytoplasmic membrane by an electron-lucent area. The ill-defined nuclear regions were surrounded by cytoplasm with densely stained ribonucleoprotein particles.

High resolution examinations were performed with phosphate buffered glutaraldehyde as the primary fixative followed by fixation with osmium tetroxide. The fine fibrils of the nucleoplasm were seen easily in the densely stained cytoplasm (Fig. 3). The cell wall was widely separated from the cytoplasmic membrane at only one end of the cell. This separation could have been a result of the centrifugal force (approx 500 × *g*) used during the preparation of the loose pellets. Steed and Murray (1966) suggested that septum formation might precede cell division in Gram-negative organisms; however, in our work the constrictive mode of division was observed in most of the dividing cells (Fig. 3*b*). Fine structure of this organism showed a morphology typical of most marine species of *Pseudomonas* (Wiebe and Chapman 1968).

The presence of lophotrichous flagella found in this study and the physiological properties of this organism reported earlier (Li and Flemming 1967) confirm our previous suggestion that this bacterium is different from *P. fluorescens*. Consultation of the literature with various known pseudomonad species leads us to conclude that this organism does not fit in any of the pseudomonad species listed in "Bergey's manual of determinative bacteriology" (Breed et al. 1957).

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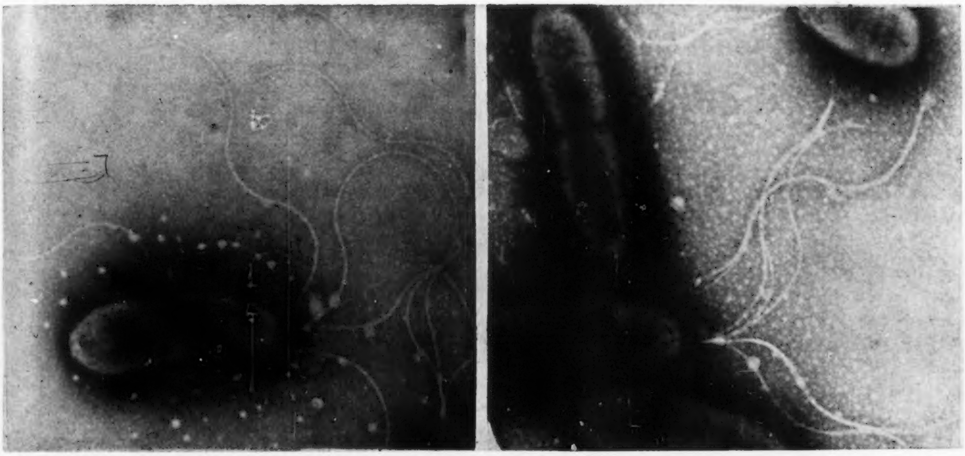

FIG. 1. Electron micrographs of negatively stained cells of the pseudomonad. Note the flagellar tuft at one pole of the cells. ×15,000.

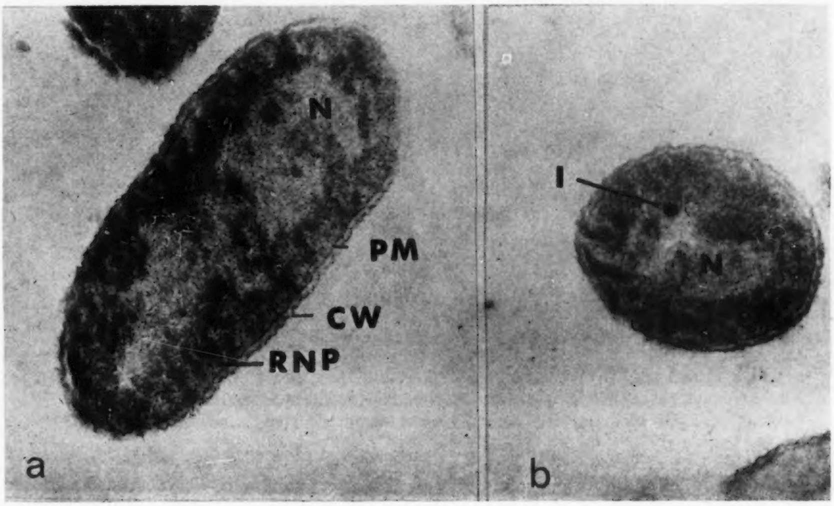

FIG. 2. Sections of the pseudomonad fixed according to the method of Kellenberger et al.: (*a*) longitudinal section, ×64,000; (*b*) cross section, ×53,000. Abbreviations for Fig. 2–3: CW, cell wall; PM, plasma membrane; N, nuclear material; I, inclusion body; RNP, ribonucleoprotein.

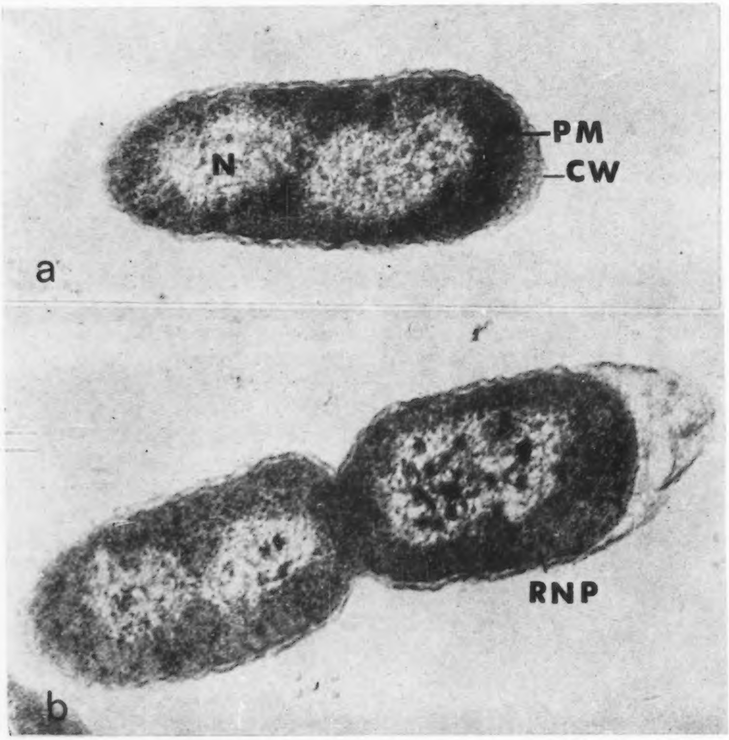

FIG. 3. Sections of cells of the pseudomonad. Glutaraldehyde and osmium tetroxide fixation, ×48,000. Note retreat of the cell plasma membrane from the cell wall. See legend under Fig. 2.

Li and Traxler — J. Fish. Res. Bd. Canada

Acknowledgements — The authors thank Dr J. E. Stewart for his interest and assistance in this work. We are grateful to Mr C. H. Castell for his critical review of the manuscript.

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Residues of DDT, DDE, and DDD in Fish in the Saskatchewan River after using DDT as a Blackfly Larvicide for Twenty Years[1]

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Fredeen, F. J. H., J. G. Saha, and L. M. Royer. 1971. Residues of DDT, DDE, and DDD in fish in the Saskatchewan River after using DDT as a blackfly larvicide for twenty years. J. Fish. Res. Bd. Canada 28: 105–109.

Concentrations of organochlorine pesticide residues in the muscle tissues of fish collected from the Saskatchewan River after a 20-year blackfly larviciding program using DDT ranged from less than 0.01–0.05 ppm of DDT, less than 0.01–0.05 ppm DDD (TDE), 0.01–0.06 ppm DDE, and 0.002–0.006 ppm dieldrin. These concentrations are well below the actionable level of 5.0 ppm of DDT residues in edible flesh. Tests with DDT-C^{14} showed that a single extraction of fish tissues with acetonitrile removed about 93% of metabolically incorporated residues.

Received August 26, 1970

In most years between 1948 and 1967 inclusive, DDT was applied once or twice to either one or both branches of the Saskatchewan River, Sask., to control blackflies (*Simulium arcticum* Malloch). The infestations of larvae requiring abatement occurred within about 100 miles immediately upstream from the confluence of these two rivers near Prince Albert (Fig. 1). The larvicide application sites and the numbers of applications at each during the 20-year period are shown in Fig. 1.

Only DDT was used; single dosages ranged from about 0.1 to 0.3 ppm sustained for 15 min in the volume of water passing the application point at the time of each application. During these 20 years totals of 2863 lb technical DDT were applied to

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[1] Contribution No. 415, Research Station, Saskatoon, Sask.

Printed in Canada (J1904)

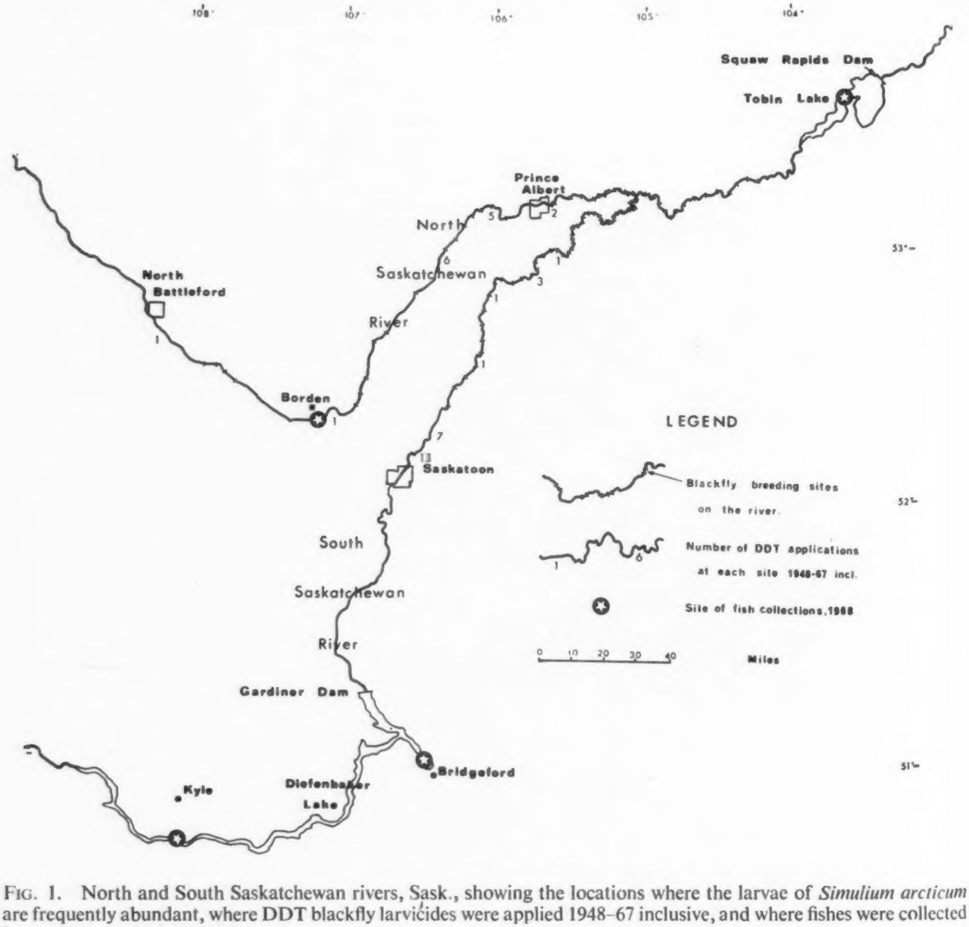
108°
107°
106°
105°
104°
53°
52°
51°
Squaw Rapids Dam
Tobin Lake
Prince Albert
North Saskatchewan River
North Battleford
Borden
Saskatoon
South Saskatchewan River
Gardiner Dam
Diefenbaker Lake
Kyle
Bridgeford
LEGEND
Blackfly breeding sites on the river.
Number of DDT applications at each site 1948-67 incl.
Site of fish collections,1968
0 10 20 30 40
Miles

FIG. 1. North and South Saskatchewan rivers, Sask., showing the locations where the larvae of *Simulium arcticum* are frequently abundant, where DDT blackfly larvicides were applied 1948–67 inclusive, and where fishes were collected for residue analyses in 1968.

the north branch and 4705 lb to the south. During this same period a total of 7.8×10^{15} lb of water flowed down both branches, providing an average dilution throughout of 0.001 parts per 10^9 of DDT in water, well below the average solubility of DDT of 3.4 ppb (Biggar et al. 1966). The amounts of DDT entering the river from other known sources, mainly annual mosquito control campaigns at Saskatoon, were probably negligible.

The purpose of this study was to compare concentrations of residues of DDT and its metabolites in the muscle tissues of fish collected from treated and untreated portions of the river.

Extraction tests with labeled DDT — The recovery of DDT from fish muscle tissue was studied by using radioactively labeled DDT.

The DDT-C^{14} (specific activity 13.7 μc/mg) was purchased from the Radiochemical Centre, Amersham, England, and shown to be at least 99% pure by thin-layer and electron-capture gas chromatography. The DDT-C^{14} was dissolved in corn oil and 5 μg equivalents of the solution (containing 68.5 mμc radioactivity) were injected into individual grasshoppers, which were fed to two rainbow trout (*Salmo gairdneri*). Each trout (30 and 47 g body weight) was fed one DDT-C^{14} treated grasshopper on alternate days for 22 days and thus received a total of 55 μg of C^{14}-DDT (753.5 mμc).

At the end of the 22 days the muscle tissues were used for recovery studies.

A 3-g sample of muscle tissue was macerated for 15 min at high speed with 20 ml of acetonitrile in a VirTis blender and filtered under suction. The filtrate was concentrated to about 5 ml in a film evaporator and made up to 10 ml. Then 1-ml aliquots were counted for radioactivity in a scintillation counter.

The residual fish tissues from the above extraction procedure were returned to the blender and macerated for a further 5 min with 20 ml acetonitrile and filtered under suction. This procedure was repeated twice and the combined filtrate concentrated to about 5 ml in a film evaporator and then made up to 10 ml in a volumetric flask. Aliquots of 1 ml were counted in a scintillation counter.

The residual fish tissues from the second acetonitrile extraction were dried at 60 C and 25-mg aliquots combusted in a Schoniger flask. The radioactive CO_2, trapped in a mixture of ethanolamine/2-methoxy ethanol (1:7, v/v), was counted by liquid scintillation.

The extraction experiment was replicated six times.

Collection of fishes from the Saskatchewan River — In early 1968, fishes were collected from four locations for determinations of residues: from the North Saskatchewan River near Borden on April 30; from the South Saskatchewan (Diefenbaker Lake) near Kyle on May 3; from the main Saskatchewan downstream from the confluence of the two rivers (Tobin Lake) on May 23; and from the South Saskatchewan (Diefenbaker Lake) near Bridgeford on June 6 (Fig. 1). Tobin Lake, a hydroelectric reservoir, is about 100 miles downstream from the confluence of the two branches of the Saskatchewan River. Since 1960 its dam at Squaw Rapids has completely prevented movements of fishes between the treated portions of the river and the extensive river–lake system downstream from the dam. Gardiner Dam, 70 miles upstream from Saskatoon, has similarly presented a complete barrier since late 1963 to the movements of fishes between the treated sections of the river and the headwaters of the south branch. Thus the fishes collected for this study from Borden and Tobin Lake can be considered to have been residents most or all of their lives in the treated portions of these rivers whereas those collected from Diefenbaker Lake will have spent the previous 4 years behind Gardiner Dam completely separated from the treated areas and thus may be considered as check samples.

The fishes were collected with nylon gillnets set for 24 hr at each site. Upon removal from the net the fishes were promptly sorted as to species, size, and sex, wrapped individually in aluminum foil, and frozen whole in solid CO_2 for transfer to the research station. There they were held at −17.8 C until analyzed. Ageing was done from scale samples.

Residue analyses — Muscle tissue was removed from each side of every fish and these subsamples were refrozen immediately at −17.8 C until used within a few days. These subsample sizes averaged about 10 g, although some of the smallest fishes yielded only about 3 g of muscle tissue. Care was taken not to include fatty tissues or major bones.

The muscle samples were macerated for 15 min at high speed with 5 ml of acetonitrile per gram of sample and filtered under suction. The residue was washed twice with 5–10 ml acetonitrile and filtered each time. The combined filtrates were then diluted with five times their volume of 1% NaCl solution and thrice extracted with 75–100 ml of petroleum ether. The combined petroleum ether layer was then washed with 100 ml of water and the water discarded. The petroleum ether extract was dried over anhydrous Na_2SO_4, concentrated to 10–15 ml, chromatographed on a 20-g alumina (grade III) column, and eluted with 250 ml of hexane: benzene (85:15) mixture. The eluent with the cleaned-up extracts was then concentrated to about 10 ml and analyzed by electron-capture gas chromatography for aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, and lindane, in addition to *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD (TDE) by injection into two gas chromatographic columns: (1) a 5 ft × 1/8 inch I.D. (inside diameter) column packed with 5% SE-30 on Chromosorb W; and (2) a 5 ft × 1/8 inch I.D. column packed with 6% QF-1 on Chromosorb W. The SE-30 column did not separate the peaks due to dieldrin and *p,p′*-DDE, or *o,p′*-DDT and *p,p′*-DDD. These compounds were, however, separated on the QF-1 column.

Extraction tests with labeled DDT — Single extractions of fish muscle tissues with acetonitrile removed an average of 92.6% of metabolically incorporated DDT-C^{14} residues (Table 1); an additional 4.5% was recovered by a second extraction with the same solvent. These results are in agreement with those obtained with pheasant tissues where about 95% of the metabolically incorporated dieldrin-C^{14} residues were recovered by acetonitrile extraction (Greichus et al. 1968). These are in contrast, however, to rather poor recoveries of dieldrin residues from plants and soils (Saha et al. 1969; Saha 1970).

TABLE 1. Efficiency of acetonitrile in extracting DDT-C^{14} residues from fish tissues

	% radioactivity in		
Expt. no.	1st acetonitrile extract	2nd acetonitrile extract	Residue (by combustion)
1	92.9	4.3	2.8
2	92.2	4.7	3.1
3	93.1	4.2	2.7
4	92.5	4.8	2.7
5	92.0	5.0	3.0
6	93.2	4.1	2.7
Avg	92.6	4.5	2.8

TABLE 2. Organochlorine pesticide residues (ppm) in the muscle tissues of fishes collected in 1968 from the Saskatchewan River, Sask.

Species	Above Gardiner Dam (near Bridgeford and Kyle)					Below Gardiner Dam (Tobin Lake and near Borden)				
	Sex and (age (*years*))	DDT	DDD	DDE	Dieldrin	Sex and (age (*years*))	DDT	DDD	DDE	Dieldrin
White sucker	2 F (4–5)[a]	<0.01	<0.01	<0.01	<0.002	6 F (3–6); 2 M (3)[b]	<0.01	<0.01	<0.01	<0.002
Longnose sucker	13 F (4–5); 4 M (4–5)[a]	”	”	”	”	5 F (6–7); 8 M (5–6)[c]	0.03	0.02	0.02	0.002
Northern redhorse	2 F (4–5)[a]	”	”	”	”	1 F (7)[c]				
Burbot	1 F (?); 1 M (?)[a]	”	”	”	”	None examined	–	–	–	–
Goldeye	3 F (3); 2 M (3)[a]	”	”	”	”	1 F (4); 1 M (2)[b]	<0.01	<0.01	<0.01	<0.002
”	–	–	–	–	–	7 F (2–3); 1 M (3)[b]	0.04	0.04	0.02	0.006
”	–	–	–	–	–	4 F (2–5); 2 M (2–4)[b]	0.05	0.05	0.06	<0.002
Yellow perch	None examined	–	–	–	–	1 F (5); 1 M (4)[b]				
” ”	–	–	–	–	–	2 F (3); 7 M (3–4)[b]	<0.01	<0.01	0.01	”
” ”	–	–	–	–	–	2 F (3)[b]	”	”	<0.01	”
Walleye	1 F (4)[d]	<0.01	<0.01	<0.01	<0.002	1 F (3)[b]	”	”	”	”
Sauger	2 ? (3)[d]	”	”	”	”	3 F (4–8); 2 M (4–9)[b]	0.05	0.01	0.04	”
Sauger	–	–	–	–	–	1 F (9)[b]	<0.01	<0.01	<0.01	”
Northern pike	1 M (3)[d]	<0.01	<0.01	0.06	<0.002	5 F (2–5); 4 M (2–3)[b]	”	”	0.01	”
” ”	1 ? (1)[d]	”	”	0.11	”	1 F (2); 2 M (2–3)[b]	”	”	<0.01	”
” ”	6 F (2–3); 8 M (2–3)[d]	”	”	<0.01	”	–	–	–	–	–
” ”	2 F (4)[a]	”	”	”	”	–	–	–	–	–

[a] Fishes collected at Kyle, May 3, 1968.
[b] Fishes collected from Tobin Lake, May 23, 1968.
[c] Fishes collected at Borden, April 30, 1968.
[d] Fishes collected at Bridgeford, June 6, 1968.

Residues in fishes from the Saskatchewan River — A total of 119 fishes of nine species were collected from the two branches of the Saskatchewan River (Table 2). These included 30 longnose suckers (*Catostomus catostomus*), 10 white suckers (*C. commersoni*), 3 northern redhorse (*Moxostoma macrolepidotum*), all bottom feeders; 21 goldeye (*Hiodon alosoides*) and 13 yellow perch (*Perca flavescens*), primarily insect feeders; 2 burbot (*Lota lota*), 2 walleyes (*Stizostedion vitreum*), 8 saugers (*S. canadense*), and 30 northern pike (*Esox lucius*), which feed on fish, crayfish, amphipods, and bottom insects. Thus attempts to obtain fish from the main trophic levels were mainly successful, although some samples were small and yellow perch were entirely absent in samples from Diefenbaker Lake.

The residue data (Table 2) are not corrected for the 93% recovery efficiency. The identities of peaks in the gas chromatogram were established by comparing retention times with those of reference compounds on the SE-30 and QF-1 columns. Most of the samples examined did not give any peaks and in the few instances where residues were indicated their concentrations were so low that they were identified only by gas chromatography.

Thirty-four fishes (17 from the two sites from the treated sections of the river and another 17 from the check sites above Gardiner Dam) were individually analyzed. Residues were detected in only two so the remaining fishes were pooled according to species and source prior to analysis. Unfortunately two of the pooled samples were of mixed species although from the same trophic levels and sources, i.e., 1 northern redhorse was included with 13 longnose suckers, and 2 perch were included with 6 goldeye (Table 2).

No discernible residues were detected in fishes collected above Gardiner Dam except in two northern pike, 1 and 3 years old, that contained 0.11 and 0.06 ppm of DDE respectively. These residues did not originate from the blackfly larviciding program because these two fish had hatched from eggs laid 2 or more years after the dam was permanently closed.

The 17 fishes collected from the treated area below Gardiner Dam, analyzed individually, contained no measurable residues. However, 54 fishes in several pooled samples from this same source were found to contain low levels of DDT, DDE, DDD, and dieldrin. The maximum combined residues of DDT plus DDE and DDD detected were 0.16 ppm in a pooled sample containing six goldeye and two perch from Tobin Lake. Even if it is assumed that only one out of the eight pooled fishes in this sample contained all the pesticide residues, the total amount of residues in that fish would have been only 1.28 ppm (cf. a tolerance of 5 ppm total DDT residues in fish). It had been expected that fishes at the end of the food chain, i.e., walleyes, saugers, and northern pike, would have had the highest levels of residues but the residue data obtained here did not fulfil that expectation.

In general these results indicate that the use of DDT in this 20-year blackfly abatement program did not result in significant accumulations of DDT or its metabolites in the muscle tissues of fishes of any trophic level in amounts that could make their flesh unsuitable as food for humans. The results also indicate that relatively small amounts of DDT were recycling in this ecosystem. However, the ultimate fate of the DDT that had been applied to these rivers was not determined. Of five other chlorinated hydrocarbon pesticides widely used in Saskatchewan in recent years for the protection of crops, only dieldrin was detected in the fish muscle tissues, and that in insignificant amounts.

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Rate at which Sockeye Salmon Alevins are Evacuated from the Stomach of Mountain Whitefish (*Prosopium williamsoni*)

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McKone, D. 1971. Rate at which sockeye salmon alevins are evacuated from the stomach of mountain whitefish (*Prosopium williamsoni*). J. Fish. Res. Bd. Canada 28: 110–111.

When adult mountain whitefish (*Prosopium williamsoni*) were force-fed two, five, or eight sockeye salmon (*Oncorhynchus nerka*) alevins in aquaria during May and June 1969, examination of the stomachs showed that the evacuation rate was 9.8–17.8% per hour, and was greater at higher than lower temperatures. Absolute rate of disappearance (grams/hour) was more rapid at higher than at lower temperatures and for larger than smaller food volumes. The evacuation rate was rapid and required only 6 hr at 11 C for complete evacuation of two alevins.

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At Babine Lake, British Columbia, during May and June 1969, observations were made of the elapsed time for evacuation from the stomach of mountain whitefish (*Prosopium williamsoni*) force-fed various numbers of sockeye salmon (*Oncorhynchus nerka*) alevins.

Methods — The basic experimental design (Table 1) was to repeat three times the feeding to 25 whitefish of two (mean weight 0.36 g), five (mean weight 0.91 g), and eight alevins (mean weight 1.33 g).

Alevins were obtained from the outfall of the Fulton River spawning channel. The whitefish were beach-seined from the Fulton River and isolated, one fish per 10-gal continuous-flow aquarium. They ranged from 22 to 34 cm and averaged between 22 and 27.8 cm in the various trials.

Each trial contained 25 fish, which were held at least 48 hr at ambient temperature. All fish used in a trial were fed two alevins 24 hr prior to an experiment. Any fish that appeared upset due to being held in captivity were replaced from reserves. Each fish was moved from

Table 1. Mean percentages of weight (blotted to remove liquid) of two, five, or eight alevins remaining in stomachs of mountain whitefish at various temperatures and times after feeding (averages of five tests of five fish each).

Date	Temp (C)	Hr after feeding											
		2	3	3.25	4	4.50	5	5.75	6	7	7.5	8	10
						Two alevins							
May 14	6	68.1			45.5				50.1			0.9	
20	8	71.5			24.9				27.4			2.34	
27	11	54.2	45.5		21.2		15.7		1.67				
						Five alevins							
May 18	8	68.2			35.2				29.6			12.7	3.90
25	11.5	65.9		40.2		20.5		7.05		3.27			
30	10.5	66.5	49.9		32.9		17.1		3.31				
						Eight alevins							
May 10	5	69.2			53.3				31.2			27.5	8.5
23	11.5	63.5			47.4				12.3		1.96		
June 7	12.5	56.1	43.7		23.4		7.03		2.45				

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its aquarium to a solution of 2-phenoxyethanol (anesthetic) and force-fed by pushing a flexible plastic tube containing the alevins down the esophagus into the stomach. A plunger followed, forcing the alevins through the tube into the stomach. The whitefish were held in aquaria and killed in groups of five, usually at 2-hr intervals, until 100% digestion occurred or until 25 fish were killed. Stomach contents were removed, blotted to remove liquid, and the solid material weighed immediately.

Results — The rate of digestion was in all instances very rapid, 100% evacuation being approached in 6 to about 10 hr. This high rate reflects either the technique used in force-feeding the whitefish or, more probably, their effective digestion process. For example, 100% digestion occurs in 24 hr at 13.3 C for Dolly Varden trout (*Salvelinus malma*) fed two sockeye salmon alevins (Armstrong and Blackett 1966); in 18 hr at 22 C for bluegill (*Lepomis macrochirus*) fed 5.5 g of natural food (Windell 1966); and in 16–19 hr at 23–26 C for largemouth bass (*Micropterus salmoides*) fed 2.5 g of *Gambusia* (Hunt 1960), and 14–16 hr at 22 C when this species was fed 5 g of minnow (Markus 1932).

The rate of evacuation was constant, showing a curvature in the logarithmic plot. Accordingly, the data were analyzed with both percent remaining and time treated arithmetically. Conditioned regression was used forcing the line of best fit through the intercept of 100% remaining at time zero. All regressions were significant, five of the nine with a nonsignificant (α = .05) mean square for curvature. In the four instances of significant curvature, the departure was in the direction that would be predicted if digestion were at a rate proportional to the amount of food remaining.

The various regressions indicate that the percentage of food remaining in the stomach declined more rapidly at higher than at lower temperatures, but at about the same rate for different volumes of food (Table 2). Thus, the absolute rate of disappearance (grams/hour) was more rapid for higher than lower temperatures and for larger than smaller volumes.

The data were not gathered so that the length of fish in relation to its evacuation rate could be considered statistically. However, some of the extremes of individual fish might be noted: for the 4-hr evacuation period, using eight alevins at 5 C, a 23.5-cm whitefish evacuated 32% whereas a 28-cm fish evacuated 57%; using the same number of alevins and evacuation rate at 12.5 C, a 24-cm whitefish evacuated 87% whereas a 28-cm fish evacuated 75%.

TABLE 2. Rates of removal of various numbers of sockeye salmon alevins from the stomachs of mountain whitefish at various temperatures. Twenty-five fish were used to establish each regression.

Mean wt (*g*)	Temp (*C*)	Weight removed per hour	
		%, from regression	g
	Two alevins		
0.38	6	11.45	0.04
0.32	8	13.17	0.04
0.34	11	17.57	0.06
	Five alevins		
0.91	8	10.93	0.10
0.90	10.5	16.90	0.15
0.91	11.5	15.34	0.14
	Eight alevins		
1.33	5	9.80	0.13
1.34	11.5	13.79	0.18
1.33	12.5	17.79	0.24

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An Undifferentiated Hematopoietic Neoplasm with Histologic Manifestations of Leukemia in a Cutthroat Trout (*Salmo clarki*)

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SMITH, C. E. 1971. An undifferentiated hematopoietic neoplasm with histologic manifestations of leukemia in a cutthroat trout (*Salmo clarki*). J. Fish. Res. Bd. Canada 28: 112–113.

An undifferentiated hematopoietic neoplasm was diagnosed in a 2-year-old cutthroat trout (*Salmo clarki*). Malignant tumor cells had invaded spleen, kidney, and liver tissue. These same cells were found circulating freely in peripheral blood, suggesting leukemia.

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WELLINGS (1969), in a recent review of the tumors of fishes, has shown that lymphosarcomas occur in several species of bony fishes and are common in northern pike, *Esox lucius*. He listed the occurrence of lymphosarcoma in three species of salmonids, which included one pink salmon, *Oncorhynchus gorbuscha*, one chum salmon, *O. keta*, one Atlantic salmon, *Salmo salar*, and several lake trout, *Salvelinus namaycush*. Most of these tumors appeared to have originated in the kidney, which in the teleost is one of the principle hematopoietic organs.

Dunbar (1969) reported lymphosarcomas in three brook trout, *Salvelinus fontinalis*, and two hybrid splake (brook trout × lake trout). He suggested that the tumors were most likely of thymic origin.

The only case of lymphosarcoma reported for the genus *Salmo* is that by Haddow and Blake (1933) for the Atlantic salmon.

This report describes the occurrence of an undifferentiated hematopoietic neoplasm suggestive of a lymphosarcoma in a cutthroat trout, *Salmo clarki*.

On March 17, 1969, a 2-year-old cutthroat trout showing exophthalmos, ascites, and a severely swollen kidney was collected from the Montana State Fish Hatchery, Arlee, Montana. Samples of midkidney, liver, and spleen were preserved in Bouin's solution and forwarded to the Fish Cultural Development Center for histological examination.

Microscopic examination of kidney tissue revealed that kidney tubules, normally quite abundant in mid-kidney, were being invaded and replaced by "blastoid" cells (Fig. 1). An occasional degenerate kidney tubule and glomerulus was observed. Trabeculae were widened, thus reducing the number of sinusoids. Cytologically, cells appeared to be primarily large, round, actively dividing stem cells. Nuclei were somewhat vesicular and a few showed chromatin margination. Occasionally, necrotic cells were observed (Fig. 2).

The spleen was composed of nodules containing cells typical of those observed in the kidney. A small amount of fibrous tissue was sometimes present in the center of larger nodules.

Numerous microscopic nodules were present within the liver, disrupting the normal architecture of the organ. The areas around portal zones and a few central veins were also invaded. Portal necrosis was common (Fig. 3).

Circulating tumor cells found in arteries and veins of all three organs examined were abundant and suggest leukemia (Fig. 4). Unfortunately, no blood smears were prepared.

Since no other organs were examined histologically, it is impossible to state with certainty the site of origin of this tumor. It would appear, however, that since there were no other grossly apparent lesions and the kidney and spleen were so extensively involved, one of these organs was the probable primary site.

The exophthalmia noted in this fish was most likely caused by orbital edema brought about by renal insufficiency resulting from invasion and destruction of renal elements.

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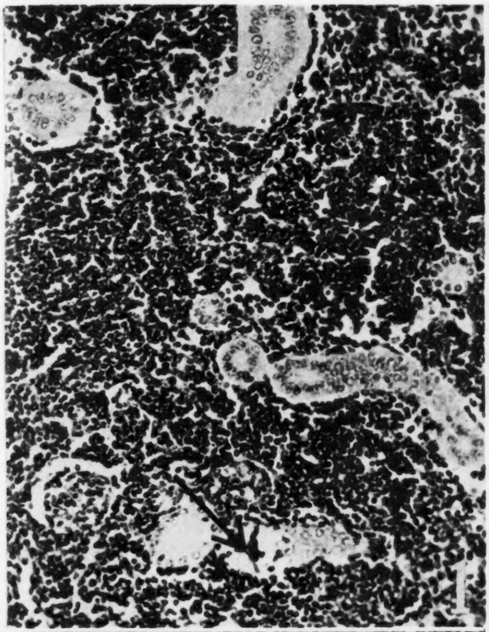

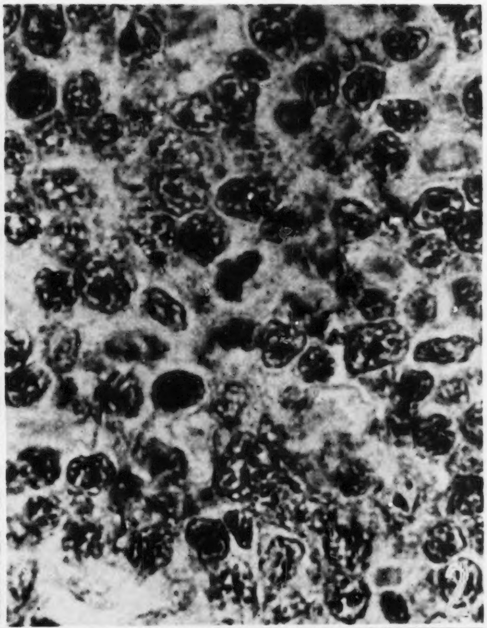

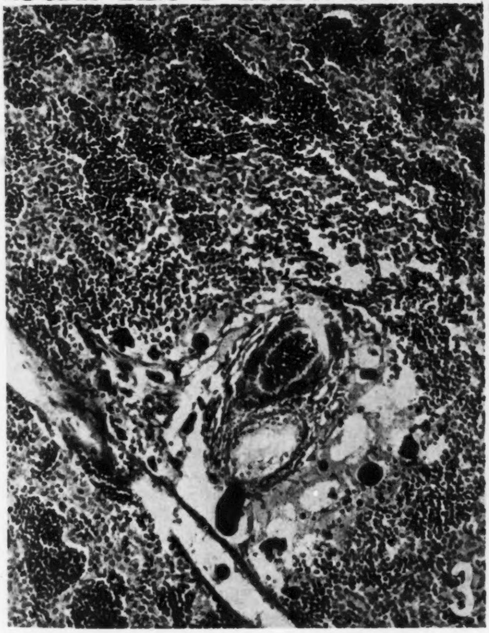

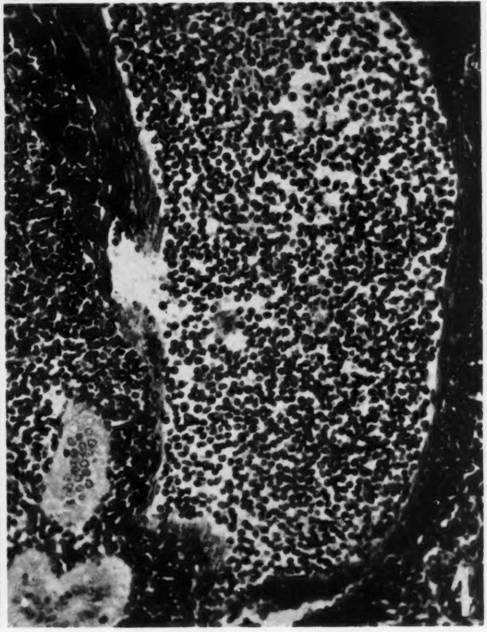

FIG. 1. Section of middle portion of kidney showing invasion and replacement of kidney tubules by malignant tissue. Note degenerate tubules (arrow). ×125.

FIG. 2. Section of spleen showing large "blastoid" cells with vesicular nuclei. Note mitotic figure in center of field. ×750.

FIG. 3. Section of liver showing portal necrosis and numerous microscopic nodules that have replaced liver tissue. ×125.

FIG. 4. Kidney section showing numerous circulating tumor cells in a large vein. ×250.

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No. 267. The author is indebted to Mr J. E. Holway, Fish Cultural Development Center, Bozeman, Montana, for collecting the tissues.

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Sampling of Fish Muscle for M.S. 222 and Quinaldine Residues

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Large variations in concentrations of M.S. 222 (tricaine methanesulfonate) and quinaldine (2-methylquinoline) residues occurred in various areas of fish fillets. Residue analyses of replicate samples from homogenized fillets yielded more representative results than samples cut from various areas of fillets.

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IN this study two sampling methods were compared to determine whether different areas of a single fish fillet contained the same concentration of residues, and which method would produce a more representative sample for analysis.

Methods and materials — Adult channel catfish (*Ictalurus punctatus*), bluegill (*Lepomis macrochirus*), and largemouth bass (*Micropterus salmoides*) were anesthetized in a 100-ppm aqueous solution of M.S. 222 at 18.5 ± 0.2 C. The desired stage of anesthesia occurred in the catfish, bass, and bluegill after 25, 35, and 60 min, respectively. The fish were then withdrawn from the anesthetic solution. One-half the number of fish withdrawn were killed instantly and labeled 0-hr withdrawal, and the other half were placed in fresh water for 1 hr and then killed.

Adult goldfish (*Carassius auratus*), bluegill, and largemouth bass were anesthetized in a 35-ppm aqueous solution of quinaldine at 18.5 ± 0.2 C. Channel catfish were anesthetized in a 75-ppm quinaldine solution. After 8 min of exposure to the drug, all fish were withdrawn and killed.

One-gram samples of tissue were analyzed for M.S. 222 residues by the colorimetric method described by Walker and Schoettger (1967a) and 10-g samples of tissue for quinaldine residues by the gas chromatographic method of Allen and Sills (1970) (Fig. 1). The standard error of the mean was determined for all groups of samples (Snedecor 1956).

Results and discussion — M.S. 222 and quinaldine residues were not homogeneously distributed throughout the muscle of fish at 0- and 1-hr withdrawal intervals. Concentrations of both residues varied far more in samples cut from localized areas of a fillet than in duplicate samples taken from the homogenized opposite fillet (Table 1). Therefore,

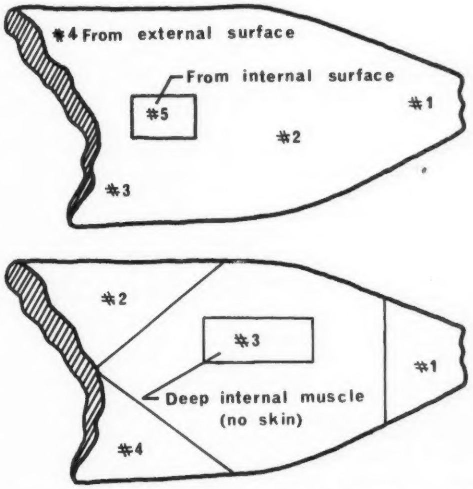

FIG. 1. Areas of the fillet from which 1-g samples (*top*) were taken for M.S. 222 residue analyses and 10-g samples (*bottom*) were taken for quinaldine residue analyses.

TABLE 1. M.S. 222 and quinaldine residues (ppm) in localized and homogenized samples of a fish fillet taken from several species of adult fish.

Species	Exposure time (*min*)	Withdrawal time after exposure (*hr*)	Residues in localized samples[a]						Residues in homogenized samples[a]		
			1	2	3	4	5	Mean	A	B	Mean
Fish treated with 100 ppm of M.S. 222 at 18.5±0.2 C											
Channel catfish	25	0	121.4	105.0	97.0	75.4	27.0	85.16±16.32[b]	43.4	44.0	43.70±0.30[b]
	25	1	11.4	6.6	16.6	6.2	4.8	9.12±2.18	7.6	8.0	7.80±0.20
Bluegill	60	0	92.0	73.4	66.0	70.4	87.0	77.76±5.00	81.0	82.0	81.50±0.50
	60	1	19.0	23.4	18.0	25.6	14.8	20.16±1.93	20.6	21.0	20.80±0.20
Largemouth bass	35	0	75.4	84.0	35.0	37.0	46.0	55.48±10.15	41.0	39.6	40.30±0.70
	35	1	9.2	9.8	10.0	10.0	10.0	9.80±0.16	13.0	12.6	12.80±0.20
Fish treated with 35 ppm of quinaldine at 18.5±0.2 C											
Goldfish	8	0	7.70	7.45	1.96	3.25		5.09±1.46	6.48	4.86	5.67±0.81
Channel catfish[c]	8	0	2.43	2.00	3.56	2.25		2.56±0.35	3.00	2.78	2.89±0.11
Bluegill	8	0	4.86	0.54	0.57	0.55		1.63±1.08	1.26	2.61	1.94±0.58
Largemouth bass	8	0	1.98	2.03	0.21	1.04		1.32±0.43	1.24	0.75	1.00±0.25

[a] Samples 1–5 cut from fillet as shown in Fig. 1; samples A and B taken from homogenized opposite fillet.
[b] One standard error of the mean.
[c] Channel catfish were exposed to a 75-ppm solution of quinaldine.

tissue cut at random from fish fillets will not yield a truly representative sample. Homogenizing the entire fillet consistently produced a more representative sample, thereby improving the accuracy of the results.

The standard error of the mean of localized samples decreased as the withdrawal time from the drug increased. Walker and Schoettger (1967b) reported that residues of M.S. 222 dissipated rapidly in rainbow, brown, brook, and lake trout within 1-6 hr after withdrawal from deep anesthesia. Unpublished data obtained at this laboratory indicated that M.S. 222 and quinaldine residues dissipated rapidly from muscle of catfish, bluegill, and bass.

Residue analyses are required to obtain clearance and registration of all new chemicals and drugs used on fish. Chemists analyzing fish for residues and biologists collecting these fish should be informed that residues can be heterogeneously distributed in fish muscle and that homogenizing the whole fish fillet will produce a homogeneous sample.

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Meristic Differences Between Anadromous and Freshwater-Resident Arctic Char (*Salvelinus alpinus*) in the Sagavanirktok River Drainage, Alaska

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McCART, P., AND P. CRAIG. 1971. Meristic differences between anadromous and freshwater-resident Arctic char (*Salvelinus alpinus*) in the Sagavanirktok River drainage, Alaska. J. Fish. Res. Bd. Canada 28: 115–118.

Both anadromous and freshwater-resident populations of Arctic char (*Salvelinus alpinus*) occur in the Sagavanirktok River drainage, Alaska. A comparison of the gill-raker and pyloric-caeca counts of populations of the two types revealed that anadromous fish had lower gill-raker and pyloric-caeca counts than freshwater-resident fish. Freshwater-resident populations correspond meristically to McPhail's Eastern Arctic form, and anadromous population scorrespond to his Western Arctic–Bering Sea form (McPhail, J. Fish. Res. Bd. Canada 18: 793–816, 1961). It is suggested that the former was the original inhabitant of the area but has been replaced by the latter except in inaccessible, headwater lakes.

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THE Sagavanirktok River and its tributaries drain the north slope of the Brooks Range (Fig. 1). The river and its major tributary, the Atigun River, are paralleled by a large segment of the proposed Trans-Alaska oil pipeline. Concern for the safety of fish populations in this drainage has prompted an investigation of the ecology of several important species, among them the Arctic char (*Salvelinus alpinus*).

Arctic char populations in the Sagavanirktok drainage are of two life-history types. First, there are lake-dwelling populations that appear to be entirely freshwater-resident, with no anadromous component. Populations in the lakes of the Campsite Drainage, in the Atigun Lakes and in Galbraith Lake are of this type. Though these lakes are not

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truly landlocked, entry from the Sagavanirktok River would not be easy because of the presence of steep, fast-water sections on the tributary streams. Second, there are populations that are almost exclusively anadromous. Such populations occur in the Sagavanirktok itself and in many of its tributaries: the Ivishak River, the Ribdon River, and in Accomplishment and Section creeks. As a preliminary to studies of the ecology of freshwater-resident and anadromous populations, the meristic characters, numbers of gill rakers and pyloric caeca were examined.

Gill-raker counts are for the first gill arch, left side, and include all rakers, fully formed and rudimentary. Pyloric-caeca counts include all caeca, regardless of size. Counts of these characters are summarized by locality in Table 1. "Campsite Drainage" and "Atigun Lakes" are the authors' designations for presently unnamed drainages. The former, a tributary of the west bank of the

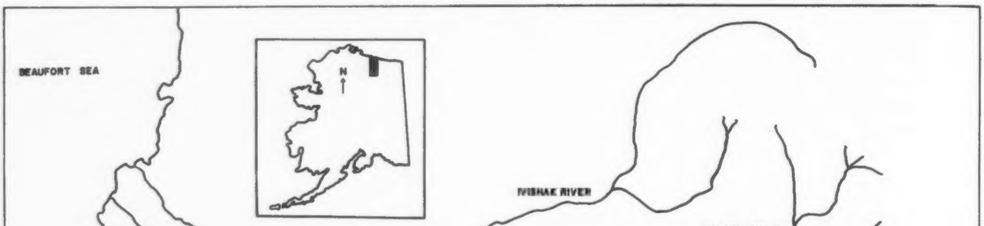
BEAUFORT SEA
N
PRUDHOE BAY
IVISHAK RIVER
RIBDON RIVER
LUPINE RIVER
ACCOMPLISHMENT CREEK
SECTION CREEK
SAGAVANIRKTOK RIVER
N
SAGAVANIRKTOK RIVER
CAMPSITE LAKES
ATIGUN RIVER
ATIGUN LAKES
GALBRAITH LAKE

FIG. 1. The Sagavanirktok River and adjacent waters.

TABLE 1. Summary of total first-arch gill-raker counts and pyloric-caeca counts for Arctic char from the Sagavanirktok River drainage. Values of *t* are for comparisons of combined samples of freshwater-resident and anadromous fish.

	Total gill rakers				Pyloric caeca			
Locality	N	Range	Mean	SE	N	Range	Mean	SE
			Freshwater-resident populations					
Campsite drainage	54	24–31	27.31	0.21	21	38–52	45.62	0.95
Galbraith	6	26–29	27.17	0.48	3	35–51	43.33	4.63
Atigun lakes	7	28–33	29.57	0.72	1	37	37.00	–
Total	67	24–33	27.54	0.20	25	35–52	45.00	0.99
			Anadromous populations					
Ivishak/Sag.	6	22–23	22.33	0.21	–	–	–	–
Ribdon	14	20–24	22.36	0.26	14	26–37	31.71	1.11
Accomplishment	42	19–27	21.73	0.23	21	23–33	28.90	0.58
Section	34	19–24	22.21	0.35	24	26–39	30.96	0.73
Total	96	19–27	22.11	0.15	59	23–39	30.41	0.46
t ($P<0.01$)			21.65				13.39	

Sagavanirktok River, drains a series of small lakes. The specimens included in the sample came from Campsite Lake itself, the largest of the lakes, and from the three lakes immediately downstream.

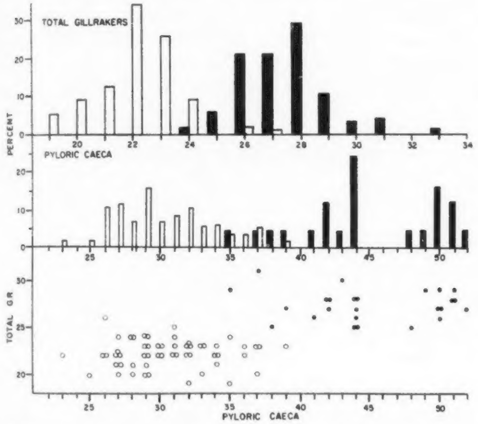

FIG. 2. Distributions of total first-arch gill-raker counts and pyloric-caeca counts for combined samples of anadromous (open columns) and freshwater-resident (solid columns) Arctic char from the Sagavanirktok drainage. Also, plots of total first-arch gill rakers against pyloric caeca for individual anadromous (open circles) and freshwater-resident fish (solid circles).

The Atigun Lakes are a group of lakes lying immediately south of Galbraith Lake. Small numbers of Arctic char were taken from these and have been combined as the Atigun Lakes sample. The sample designated "Ivishak/Sag." was taken at the juncture of the Ivishak and Sagavanirktok rivers and could not be assigned to either stream.

The anadromous and freshwater-resident populations differed markedly in both meristic characters. The former had consistently lower gill-raker and lower pyloric-caeca counts than the latter. There was some overlap in the total distributions of both counts, but modes were distinctly separate (Fig. 2). Plots of gill rakers against pyloric caeca for individual fish reveal two nonoverlapping groups of points corresponding to the two life-history types (Fig. 2). Within these groups, there is no significant correlation between the two characters. The calculated correlation coefficients for the regression of gill rakers on pyloric caeca were $r = 0.024$ for the anadromous Arctic char and $r = -0.063$ for the freshwater-resident char.

McPhail (1961) has also examined meristic variation in Arctic char. He surveyed samples from throughout the North American range of the species and was able to distinguish three geographic groups based primarily on differences in gill-raker and pyloric-caeca counts: (1) an Eastern Arctic form continuously distributed from Labrador to a point east of the Mackenzie River, (2) a Western Arctic–

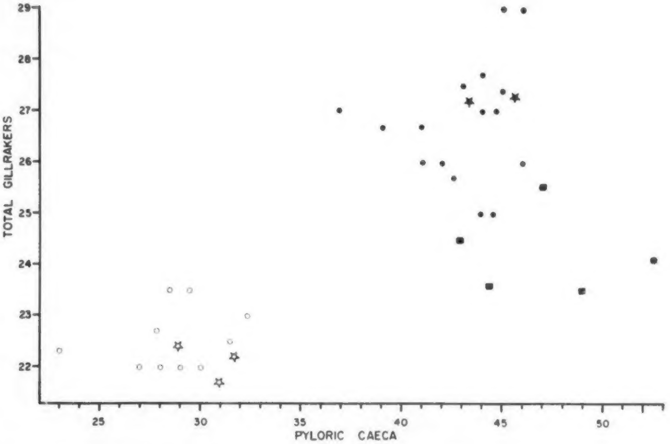

FIG. 3. Plots of population means for total first-arch gill rakers against population means for pyloric caeca: open stars, Sagavanirktok River anadromous populations: solid stars, Sagavanirktok River freshwater-resident populations; open circles, Western Arctic—Bering Sea populations; solid circles, Eastern Arctic populations; solid squares, Bristol Bay—Gulf of Alaska populations. Data for last three groups from McPhail (1961). Only means based on samples of two or more fish have been included.

Bering Sea form distributed from the Mackenzie River to the Kuskokwim River, and (3) a Bristol Bay–Gulf of Alaska form south of the Kuskokwim River. McPhail suggested the latter is a remnant of the Eastern Arctic form whose distribution, once continuous across North America, was interrupted by glaciation, possibly the Wisconsin.

When the mean gill-raker and pyloric-caeca counts for individual Sagavanirktok River populations are plotted along with mean counts for populations examined by McPhail (Fig. 3), it is apparent that the Sagavanirktok River anadromous populations correspond meristically to McPhail's Western Arctic–Bering Sea form whereas the Sagavanirktok freshwater-resident populations correspond to his Eastern Arctic form.

McPhail states that the Eastern Arctic form occurs as both anadromous and freshwater-resident populations though, in the Sagavanirktok drainage, they appear to be exclusively the latter. The present distribution of the Eastern Arctic form in the upland lakes of the drainage suggests that they may have been its original inhabitants. One possibility is that the Western Arctic–Bering Sea form was better adapted to the anadromous mode of life and displaced the Eastern Arctic form in the lower reaches of the Sagavanirktok and its tributary spawning streams. The latter survive only in areas that, because of difficulty of access or for other reasons, are unsuitable for anadromous fish.

McPhail suggests that the Eastern Arctic and Western Arctic–Bering Sea forms may warrant subspecific designation. The fact that they exist as distinct entities in the same river drainage tends to reinforce this opinion. Conceivably, they are distinct species. However, as McPhail points out, any further nomenclatural complication is best avoided until the relationship between Eurasian and American forms of *S. alpinus* can be determined.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles have been included in an annual volume of "Studies," composed of two parts. The final binding is the 1969 Studies, Part 2, which included No. 1355. These will no longer be bound and distributed. We will continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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